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CO-EXTRUSION AS A CONTINUOUS PRODUCTION PROCESS FOR FIXED-DOSE COMBINATION DOSAGE FORMS

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Ghent, April 20th, 2015

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LIST OF ABBREVIATIONS

API	active pharmaceutical ingredient
ATR FT-IR	attenuated total reflectance Fourier transform infrared
BMP	bitmap
CCD	charge coupled device
DBS	dibutyl sebacate
DOE	design of experiments
DSC	differential scanning calorimetry
EC	ethylcellulose
ER	extended release
EVA	ethylene vinyl acetate
FDA	Food and Drug Administration
FDC	fixed-dose combination
GIT	gastrointestinal tract
GMP	Good Manufacturing Practice
GRAS	Generally recognized as safe
HCT	hydrochlorothiazide
HME	hot-melt extrusion
HPLC	high performance liquid chromatography
IR	immediate release
LDPE	low density polyethylene
MDSC	modulated differential scanning calorimetry
Micro-CT	micro-computed tomography
MPT	metoprolol tartrate
NDA	new drug application
NIR	near infrared
NSAID	non-steroidal anti-inflammatory drug
PAT	process analytical technology

PEG	polyethylene glycol
PEO	polyethylene oxide
RCS	refrigerated cooling system
ROI	region of interest
SNV	standard normal variate
SPE	solid phase extraction
TEC	triethyl citrate
T_g	glass transition temperature
TPI	terahertz pulsed imaging
TSE	twin screw extruder
UHPLC	ultra high performance liquid chromatography
USP	United States Pharmacopeia
UV	ultraviolet
XRD	X-ray diffraction
WHO	World Health Organization

OUTLINE AND OBJECTIVES

Co-extrusion is defined as the simultaneous hot-melt extrusion of two or more materials through the same die, creating a multilayered extrudate. The technique allows to combine the desirable properties of multiple materials into a single structure with enhanced performance characteristics. It is an innovative technology for the continuous production of multilayered dosage forms that offers numerous advantages over traditional pharmaceutical processing techniques. Co-extrusion provides great potential for the production of fixed-dose combination products, which are gaining importance in pharmaceutical industry due to their improved patient convenience and adherence and better clinical outcomes. The **objective of this work** was to examine the possibilities of hot-melt co-extrusion for the production of multilayer oral dosage forms, providing different release profiles for each of the drugs incorporated in the core and coat of the co-extruded form.

In the **Introduction** hot-melt co-extrusion is reviewed as a manufacturing technology, illustrating its advantages and shortcomings. An overview of the equipment required for co-extrusion is provided, including the downstream solutions to process a final dosage form. Medical and pharmaceutical applications of the technology and proper material selection are pointed out. The increasing importance of fixed-dose combinations in the pharmaceutical industry is discussed.

In **Chapter 1**, hot-melt co-extrusion is evaluated as a technique for the production of fixed-dose combination mini-matrices, using an ethylcellulose core to control the release of metoprolol tartrate and a polyethylene oxide-based coat to obtain immediate release of hydrochlorothiazide. The *in vitro* performance of the different formulations was assessed. The physicochemical state of the drugs in the formulations was characterized using modulated differential scanning calorimetry (MDSC), X-ray diffraction (XRD) and Raman spectroscopy. Furthermore, the physical stability of the co-extruded mini-matrices was monitored during 6 months storage at 25 °C/60 %RH and 40 °C/75 %RH. The bioavailability of the different formulations was evaluated after oral administration to dogs and compared to that of a commercially available fixed-dose combination product. Additionally a mathematical model considering the controlled release of metoprolol tartrate from the dosage form and the *in vivo* fate of the drug was developed and used to predict the resulting drug plasma profiles, based on the *in vitro* results and *in vivo* parameters reported in literature.

In **Chapter 2**, calendering is used as a downstream technique to shape the co-extruded fixed-dose formulation in a continuous way into its final form. Co-extrudates with a metoprolol tartrate-loaded sustained-release core and a hydrochlorothiazide-loaded immediate-release coat were immediately shaped into a monolithic drug delivery system via calendering, using chilled rolls with tablet-shaped cavities. The dosage forms produced were evaluated for *in vitro* drug release, coat thickness, uniformity and pore structure. The impact of the calendering step on the physical state of the drugs in the formulations was characterized using MDSC and XRD.

The aim of the experiments performed in **Chapter 3** was to design a fixed-dose combination dosage form which provides sustained release for both the freely water-soluble metformin HCl and the poorly soluble gliclazide, two anti-diabetic compounds used to treat diabetes mellitus, using co-extrusion as manufacturing technique. Developing a matrix formulation that sustained metformin release was challenging, given that its high dose requires a high drug load in the formulation and that the drug is freely soluble. Both active pharmaceutical ingredients (API's) were formulated in a single dosage form composed of two separate layers, each demonstrating adequate properties for the incorporated API. *In vitro* release and physicochemical state of the drugs incorporated was characterized.

In **Chapter 4**, hot-melt co-extrusion is used as a processing technique to manufacture a fixed-dose combination product providing enteric protection to naproxen incorporated in the core and immediate release to esomeprazole magnesium embedded in the coat. Both core and coat were first independently developed. Several enteric polymers (Eudragit® L100-55, HPMC-AS-LF and HPMCP-HP-50) were tested as core matrix former in combination with naproxen. The physicochemical state of the drug in the extrudates was determined and a stability study was performed. Intermolecular interactions between naproxen and polymer were identified using attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectroscopy. Since gastro-protective co-therapy using a proton pump inhibitor is recommended to decrease the incidence of non-steroidal anti-inflammatory drug (NSAID)-related adverse events, esomeprazole magnesium was formulated in a separate non-enteric polymer layer providing immediate drug release. Different polymers were tested and their influence on release and physicochemical state characteristics was monitored. Finally it was evaluated if co-extrusion of a core/coat dosage form allowed to formulate the two

chemically incompatible API's in a fixed-dose combination that offered the desired release profile for both API's.

INTRODUCTION

HOT-MELT CO-EXTRUSION: PROCESS AND APPLICATIONS

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ABSTRACT

In this chapter co-extrusion is reviewed as an innovative continuous production technology for fixed-dose combination drug products. The equipment needed for co-extrusion of pharmaceutical dosage forms is summarized. Because the geometrical design of the die dictates the shape of the final product, different die types are discussed. As a major challenge is shaping of the co-extruded formulation into its final form via a continuous process, an overview of downstream solutions for processing co-extrudates into drug products is provided. Important requirements for material selection are pointed out. Examples of medical and pharmaceutical applications are presented. Co-extrusion offers great potential for the continuous production of fixed-dose combination products which are gaining importance in pharmaceutical industry, since combination therapy is increasingly recognized as a major advantage, not only for life cycle management of drugs but also for therapeutic reasons. Although co-extrusion is a very promising formulation technique, there are still some barriers to the implementation of co-extrusion in the pharmaceutical industry. The optimization of downstream processing remains a point of attention.

INTRODUCTION

HOT-MELT CO-EXTRUSION: PROCESS AND APPLICATIONS

HOT-MELT CO-EXTRUSION

Hot-melt extrusion (HME), a processing technique widely used in plastic and food industry, is defined as the pumping of raw materials through a die into a product of uniform shape by means of a rotating screw and under elevated temperature [1]. HME is a viable method for the production of granules, pellets, spheres, tablets, capsules, implants and transdermal, transmucosal or transungual films [2, 3]. This technique has found its way into the pharmaceutical industry because of its advantages over conventional techniques, such as the possibility to improve drug solubility, to sustain drug release and to mask bitter taste of an active pharmaceutical ingredient (API). Other major benefits of the technique are that there are no requirements for the compressibility of the formulation, the continuity of the process (requiring fewer processing steps) and the low environmental impact (given the solvent-free nature of the process) [1-5]. Continuous manufacturing also provides specific advantages compared to a batch-wise approach: a shorter development time because of a more straightforward scale-up, easier automation of the production line, improved quality and less product variability (via in-line quality control), a faster product release, lower product rejection rates [6]. The reduction in production costs when shifting to HME as a continuous

processing technique may however be nullified by the investment in novel equipment and by the need for experienced line operators, given the increased level of process complexity. HME is now being used in pharmaceutical industry for the production of drug delivery systems like transdermal patches and solid dosage forms. The number of HME patents issued worldwide also importantly increased since the early 1980s [3].

When hot-melt extrusion of two or more materials is performed through the same die, to create a multiple layer extrudate, the process is defined as co-extrusion [7]. In this way the properties of multiple materials are combined into a single structure. The technique has already been used for several decades in plastics and food industry to produce a broad range of applications, e.g. pipes, wires, packaging material and filled snacks. Co-extrusion has extensively been used to produce medical devices, but is relatively new for pharmaceutical applications. However, several applications of co-extrusion for oral drug delivery are possible: (a) combining drugs with different release profiles since drug release can be individually modulated, depending on the characteristics of the layer in which the drug is incorporated, (b) producing a solid dosage form which provides dual release of a single drug, (c) simultaneous administration of non-compatible drugs.

PROCESS AND EQUIPMENT

Co-extrusion implies extruding two or more materials through a single die. The materials for each of the layers (API, polymer, plasticizer and/or other additives) are premixed or separately fed into an extruder. In each heated extruder barrel the material is softened, mixed and finally extruded through the die, where the different melt streams are combined into the final co-extrudate. The co-extrudate is then shaped, cooled and further processed. Throughout the entire process several important process parameters need to be controlled. Process analytical technology (PAT) can be used for in-line control of the product quality. The co-extrusion equipment that was traditionally designed for the plastics industry needed to be adapted to meet regulatory requirements for pharmaceutical use. All product contact parts need to be Good Manufacturing Practice (GMP)-compliant. Pharmaceutical design also includes perfect cleanability, process reproducibility proven by stringent documentation and the use of Food and Drug Administration (FDA)-approved materials. Another challenge is the miniaturization of pharmaceutical extrusion equipment, in particular for the development of formulations with new chemical entities [8]. While there is no need for a special co-extrusion design of the upstream equipment (feeders and extruders), the specific requirements for die and downstream equipment with regard to co-extrusion will be discussed. All equipment of a co-extrusion line needs to fit together and the extruders have to be positioned in a way that they can easily be connected at the die (Fig. 1). Therefore the overall design of the co-extrusion line, fit for the dimensions of the production facility, is important.

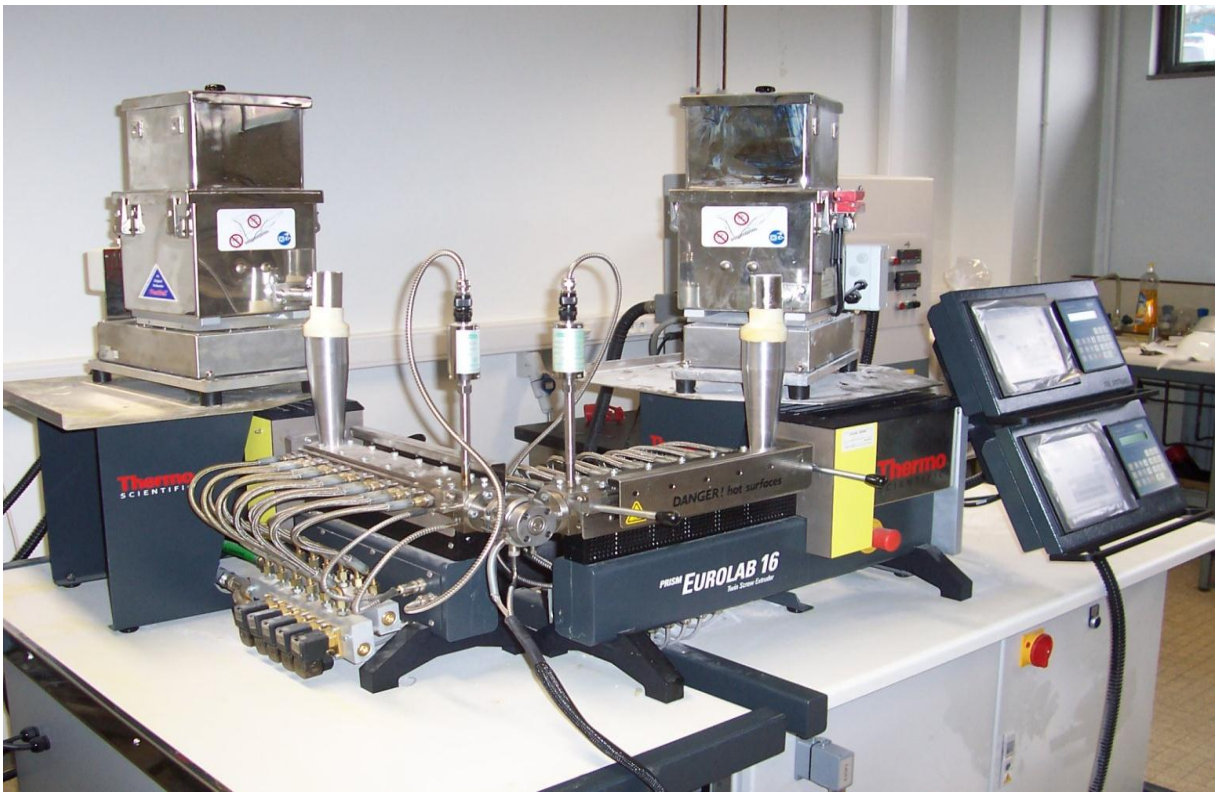

Figure 1. Example of a co-extrusion line.

Feeders

In co-extrusion the material mix of each layer is fed separately into the barrel of an extruder. Materials for each specific layer can be either premixed in a fixed ratio or individually metered into the extruder. Feeding in a constant and accurate way is a challenging but very important aspect in pharmaceutical extrusion processes. Feeding is either starve-fed, where the rate is set by the feeders, or flood-fed where the extruder screw speed determines the output.

Powders are mainly fed into the extruder using screw feeders which can be optimized by choosing the type of screws according to the powder characteristics. For powders with poor flow properties co-rotating twin screw feeders can be used instead of single screw feeders. An improved hopper design and discharging aids can also be built into the feeders to avoid bridging or other feeding problems. Feeders are controlled in a gravimetric or volumetric

way. The controller of a volumetric feeder imposes a constant rotation speed, which can result in high mass-flow fluctuations, whereas a gravimetric or loss-in-weight feeder monitors the weight fluctuation per time interval and modifies the rotation speed to keep the mass-flow rate constant. It is obvious that loss-in-weight feeders are typically preferred in pharmaceutical GMP installations.

Extruders

Extrusion processes can be categorized as either ram or screw extrusion. In ram extrusion high pressures are applied to displace a ram in order to push the heated material through a die. Screw extrusion uses one (single screw) or two (twin screw) screws to transport the material. Screw extruders are preferred over ram extruders since they provide more shear and intense mixing, resulting in a better homogeneity and temperature uniformity. The single screw extruder is the most widely used extrusion system in the plastics industry, while twin screw extruders (TSE) are preferred for pharmaceutical applications because of their high kneading and dispersing capacities, short residence time and -in case of intermeshing machines- their self-wiping sanitary screw profile [5]. TSE's are starve-fed, which means the feed system determines the output rate. The screws of a twin screw extruder can be either co-rotating or counter-rotating [1]. In pharmaceutical industry the intermeshing co-rotation mode is preferred, since it provides intensive mixing and ensures almost complete emptying of the extruder, minimizing loss of highly valuable product. These extruders operate by a first in - first out principle and minimize the non-motion, thus preventing localized overheating of materials within the extruder. These advantages indicate that this type of extruders is the best option for pharmaceutical co-extrusion.

The three basic functional screw element types are classified as forwarding, mixing and zoning. Forwarding elements are usually flighted, and they convey material from the feed opening towards the die. Mixing elements can be dispersive or distributive. In distributive mixing individual domains are unchanged, in dispersive mixing morphological units are broken down by shear and elongation. Mixing elements may have a balance of both properties. Furthermore mixing elements can be forwarding, neutral or reversing, the latter changing the direction of material flow by pushing the material backward. Zoning elements can be used to separate unit operations [9].

Based on the design and function of the screws an extruder is typically divided into three sections along its length. In the feeding section the material will be transferred from the hopper to the barrel. Once the material enters the compression section it will begin to soften or melt. The temperature of this section is normally set at 30 - 60 °C above the glass transition temperature of amorphous polymers or the melting point of a semi-crystalline polymer [10]. This can be used as a rule of thumb although exceptions have been described [11, 12]. Of course intermolecular interactions also determine plasticizing or anti-plasticizing effects. In this section the mixing elements will perform their dispersive and/or distributive mixing operation. The molten material finally enters the metering section, where the pulsating flow is reduced to ensure a uniform delivery rate through the die cavity. The output rate of the extrudate is highly dependent on the channel depth and the length of this metering section. Especially in co-extrusion it is important to make sure that the design of the screws is ensuring a stable throughput. Sometimes a melt pump is used to reduce the pressure and throughput instability, called melt pulsation [13]. Apart from this technological solution, melt pulsation can also be prevented by constructional screw and barrel adaptations such as changing the length, diameter and pitch of the screws [14]. Mounting

intensive mixing elements results in a significant improvement of the homogenization of the processed material. In combination with special densification elements at the end of the screw melt pulsation can effectively be eliminated.

Dies

Before exiting the extruder the melt is pumped through a die, which is mounted at the end of the barrel, and is hereby exposed to high pressure. The geometrical design of the die will dictate the shape of the final product [10]. In co-extrusion the die design is crucial for shaping co-extrudates with the desired characteristics. A lot of different designs are possible, but for every design the rheological behavior and temperature distribution of both melts need to be modeled accurately. Another important aspect of co-extrusion is the contact surface between the materials, dictated by the shape of the co-extrudate, and the contact time of the materials in the die, which is illustrated by the difference between single- and multi-manifold dies.

Figure 2. Schematic representation of a multi-manifold die and a single-manifold die.

Two basic die types used in co-extrusion systems are multi-manifold dies and single-manifold dies (Fig. 2). Multi-manifold dies exhibit individual manifolds for each layer and each manifold is designed to distribute its polymer layer uniformly before combining with other layers. In most cases the layers are combined inside rather than outside the die in order to

prolong the thermal contact period and thus improve the interfacial adhesion between the layers. Since in a multi-manifold die the different layers are quite established prior to combination, migration is minimized and thus a very uniform distribution of layers is achievable, which is a major advantage for co-extrusion in pharmaceutical applications.

In a single-manifold die only one manifold is present. In plastics it is often combined with a feedblock. In a feedblock with single-manifold die design a multilayer composite is formed in a combining adaptor prior to delivery to a flat die. The feedblock arranges the incoming melt streams in the proper sequence and balances the velocities of the components. The multilayer composite is then compressed into a rectangle and delivered to a flat die where the composite is spread and thinned to its final form. Currently there is a trend to more co-extruded layers with micro-layer structures containing up to ten layers of a thickness down to 1 micron produced by multilayer co-extrusion dies [15]. One of the most important considerations in feedblock co-extrusion is layer uniformity. Layer non-uniformity can be corrected by feedblock profiling in order to shape the polymer composite prior to entering the die [16].

The classification of dies is not only made by the distinction between single- and multi-manifold, they can also be classified by their shape. Flat dies (Fig. 3) convert materials from the circular shape at the end of the barrel into a thin wide sheet and are thus suitable to produce films and lamination systems. These are used for packaging materials and for transdermal and dissolvable film applications.

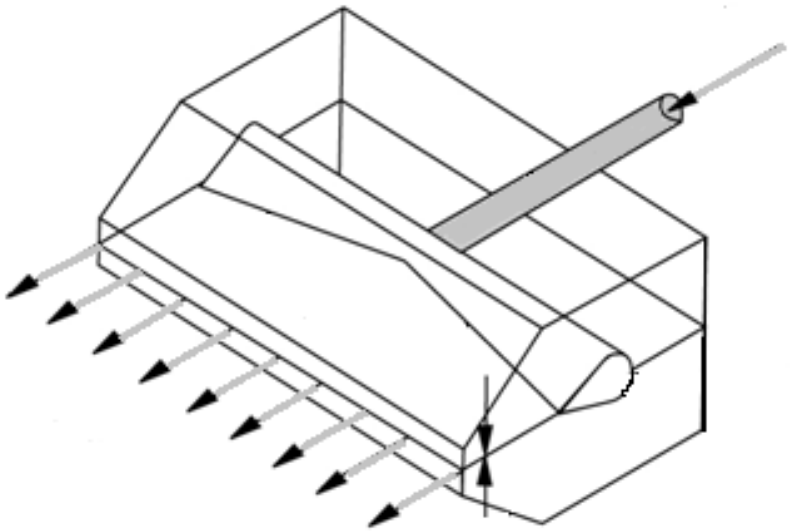

Figure 3. Example of a flat die.

Since the material path along the centerline is shorter in comparison with the one at the edges these dies need a design that prevents the sheet from being thicker in the center than at the edges in order to achieve a uniform distribution of material across the width [17]. When the polymer melt is extruded through a slit die onto highly polished cooled rolls which form and wind the finished sheet, the process is known as cast film extrusion. When the melt is extruded vertically into a tubular film and inflated by air, the process is known as blow film extrusion [5].

For medical device applications (e.g. tubings) annular dies are used. Side fed dies and spiral mandrel dies (Fig. 4) are used in applications where a substrate needs to be coated or in co-extrusion.

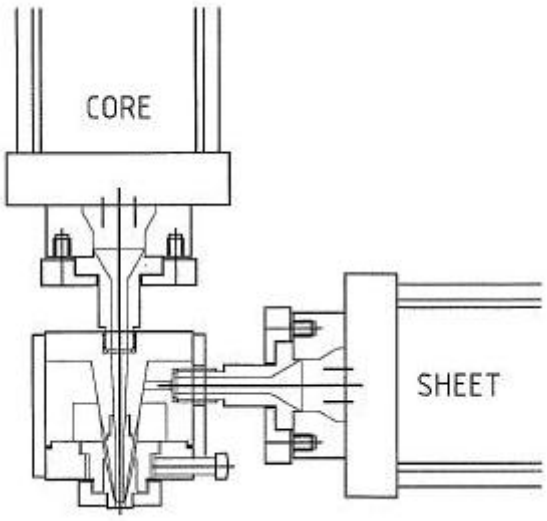

Figure 4. Thermo Fisher Scientific® co-extrusion spiral mandrel die with cylindrical core and annular coat. (Reprinted with permission of Thermo Fisher Scientific®)

The mixing effect of the spiral distribution system provides improved product uniformity, which is very advantageous for pharmaceutical applications. For the design of a die the thickness uniformity, residence time and residence time distribution are important criteria. In a spiral mandrel type die the material is exposed to a larger residence time distribution, which can cause degradation because of the longer exposure to the processing temperature in the die [18].

Two exit phenomena that can occur when the processed material leaves the co-extrusion die, need to be considered: extrudate swell and sharkskin. These phenomena are common in polymer processing, but need extra attention in co-extrusion as they can occur in each of the co-extruded layers.

Extrudate swell, also known as die swell, is a situation where the diameter of the extrudate increases upon exiting the die. The polymer melt is compressed when entering the die, followed by a partial recovery (i.e. swell) to the former shape and volume after exiting the die. It is an entropy-driven phenomenon that occurs when the individual polymer chains,

due to their viscoelastic properties, recover via relaxation from the deformation caused by the rotating screw inside the barrel. The extent of extrudate swell depends on external factors as well as on factors intrinsic to the polymer [19].

The other exit phenomenon, sharkskin, is an extrudate surface defect characterized by small scale and high frequency roughness. As an explanation for its occurrence it was claimed that sharkskin results from the cracking of the fluid at the die exit, under action of the high tensile stresses which may develop in this zone [20].

Downstream processing equipment

Currently several downstream solutions for shaping extrudates into oral dosage forms are used in pharmaceutical industry. When extrudates are milled and subsequently tabletted or filled into capsules, the process is discontinuous. In pharmaceutical applications, and more specific in co-extrusion, one of the major challenges is shaping the final product in a continuous way. The following techniques have already been applied in hot-melt extrusion and could be extended to co-extrusion.

Cooling and conveying

Chill rolls are used as an intermediate process step, to cool down and control the temperature of extruded films. Two rolls with a defined slit opening, temperature and speed create a temperature gradient throughout the extrudate to cool it in a controlled way. Highly polished rolls that apply a very well controlled cooling are advantageous to maintain product characteristics and can immediately process thin extruded sheets. If the extruded material is brittle the sheets will form flakes on an agitated conveyor belt. These flakes need to be milled or crushed, and afterwards the powder can be used for tableting or capsule filling.

Controlling the cooling rate is of major importance when the amorphous nature or crystallinity of the API has an impact on the in-vivo performance of the drug. Film thickness can be adjusted by changing the rotating speed of the chill rolls. A rod shaped co-extrudate can be conveyed using a conveyer belt and cooled in a water bath or by a cooling ring or air tunnel, before further processing.

Pelletizing

Using a pelletizing/cutting system co-extruded strands can be turned into small pellets (down to 0.5 mm) in a continuous way. These pellets can be milled, filled into capsules as such or further modified by spheronization [21].

A traditional pelletizer consists of blades on a helical rotor. A drawback of this system is that uniform pellets with constant dimensions can only be obtained for rigid materials, as extrudates composed of soft material will yield deformed pellets. In continuous mode a high throughput rate can be obtained, but the edges of the pellet are not straight. When cutting in a start/stop mode a lower throughput rate and straight edges can be obtained. Challenges are abrasion of the cutting blades and clogging of material. Teflon-coated knifes can be used to avoid clogging effects, while an air pressure nozzle can avoid accumulation of the pellets in the cutting zone. These improvements will tackle the current challenges and offer a widely applicable pelletizing system.

Although spheronization of pellets at elevated temperatures in a traditional spheronizer has proven to be a successful processing step [22, 23], currently die face cutters are available post-extrusion for the production of spheronized micro-pellets. The strand is cut immediately after it leaves the nozzle. Spheronization occurs as a result of die swelling. Recently a novel die has been designed to include a direct pelletization/spheronization step.

Cutting of the hot, still molten strand has been achieved with a rapidly rotating cutter knife. The granules instantly form perfectly shaped micro pellets due to the action of surface tension and the shrinkage due to solidification. The importance of a proper temperature control of the extrusion die has been shown by Radl et al. [24].

In case of annular shaped co-extrudates a traditional type of pelletizer is less suitable, since the inner layer of the extrudate may be pushed out of the outer layer. Cutting of co-extrudates requires a punching system or a pelletizing system with a very sharp knife in order not to put too much pressure on the inner layer and to obtain pellets with constant composition and dimensions. Therefore this technique is not ideal for co-extrudates composed out of hard or brittle materials. Co-extrudates cut into small mini-tablets have open sides, which has an influence on the release pattern of the inner layer [11].

Calendering

Calendering is the downstream solution where the molten co-extruded strand is forced between temperature-controlled rolls to produce sheets that already contain single tablet cores. Calendering is a further step towards a continuous process by allowing immediate final shaping of the extrudate by calendar rolls that contain tablet- or pill-shaped cavities. This technique is already used in the plastics or confectionary industry in order to produce monolithic shapes. In pharmaceutical applications the Meltrex® technology offers calendering as one of the possible solutions to shape the molten strand, immediately after leaving the extruder [25]. There is always some waste material at the sides of the tablets or pills, which is a disadvantage when working with highly valuable active ingredients, but the continuous on-line shaping of the product via calendering is a major advantage over pelletizing, where a subsequent process step is inevitable to obtain a final dosage form.

In case of co-extrusion calendering can be a good solution since the shaping by the rolls will make sure that the outer layer is entirely surrounding the inner core (Fig. 5). Yet, it has to be mentioned that for drug delivery systems where the coat steers the drug release rate from the core, it is challenging to obtain a coat that completely occludes the core by means of calendering since it is difficult to produce a completely sealed outer layer via this technique. Nevertheless calendering has an advantage over pelletizing of the co-extrudates where both sides are open and the inner layer of the co-extrudate is exposed.

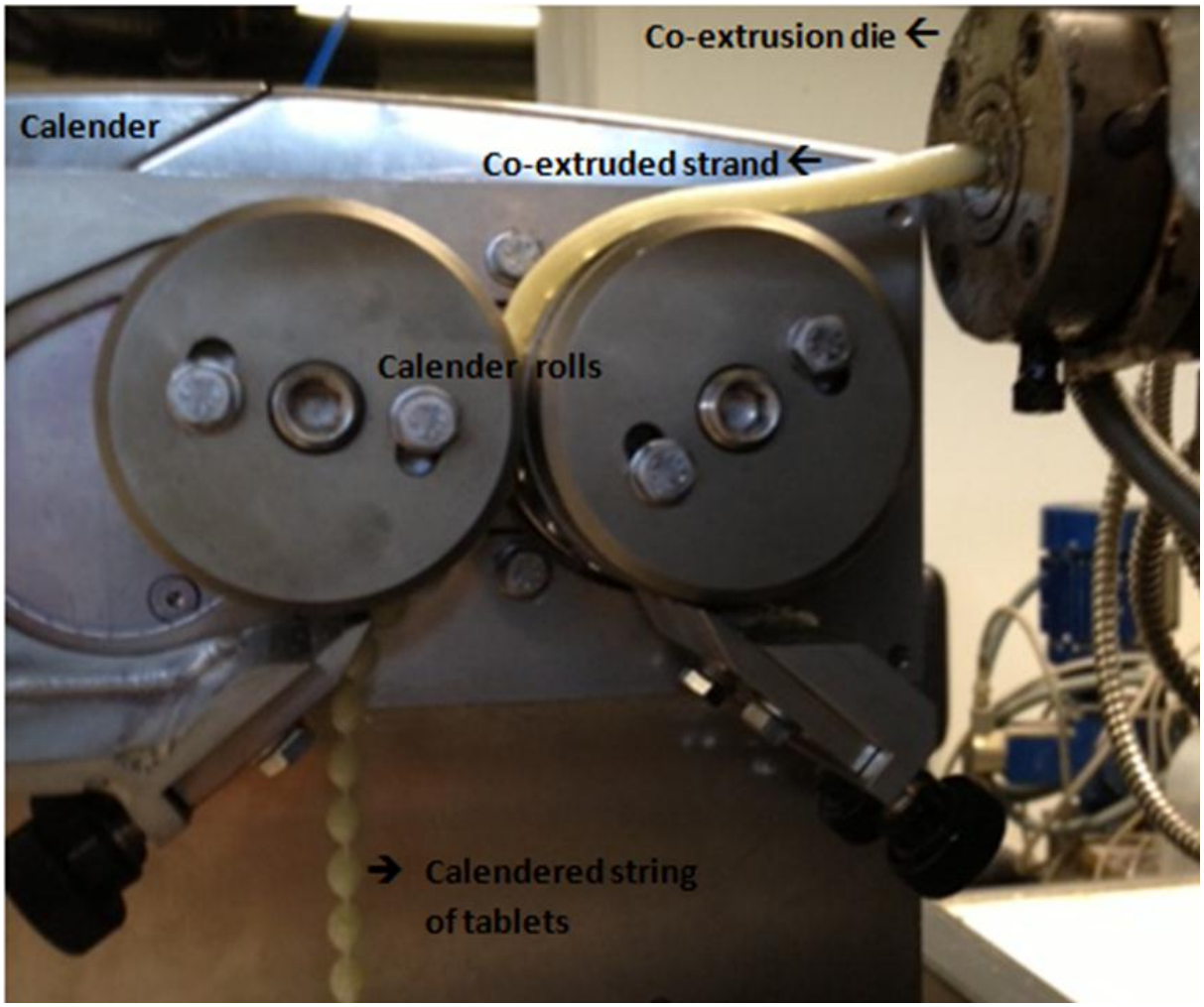

Figure 5. Example of calendering in co-extrusion.

Injection and blow molding

During injection molding the molten plastic is injected into a cavity mold at high pressure. The material cools and solidifies in the cavity. The final product is removed from the cavity after the mold has been opened. This technique is the most reliable option to obtain final dosage forms with a unique shape, perfectly matching the specified dimensions. It was transferred from the plastics industry and can be used in the pharmaceutical industry for the production of conventional and controlled-release dosage forms. It can be used as an economically advantageous manufacturing technique or as an approach to new drug delivery systems [26]. Quinten et al. demonstrated that injection molding of thermoplastic pharmaceutical formulations based on ethylcellulose (EC) and polyethylene oxide (PEO) is a promising technique to prepare sustained release matrix tablets [27]. Vaz et al. have shown that co-injection molding is a successful way of producing a double layer core/coat drug delivery device which offers a lot of opportunities for processing of co-extrudates [28]. Compared to calendering the higher equipment cost for the two-step co-injection molding process is the major drawback. Co-injection molding is commercially used for the manufacturing of the Egalet® technology (cfr. page 28).

Co-extruded tubing can be shaped by means of blow molding, mainly used for the production of containers. The tubing is extruded into an open mold. After the mold has been closed, compressed air is blown into the open end of the tube, thus expanding the viscous material to the walls of the cavity, forming it into the desired shape of the container. When a product has specific shape or dimension requirements, molding is the best alternative as a downstream processing technique.

Process monitoring and control

Variables that need to be controlled throughout the entire process are feed rates of the ingredients, barrel and die temperatures, motor speed and specific parameters for the downstream equipment, e.g. extrudate diameter control by a laser gauge. Parameters that need to be monitored are actual feed rate and temperatures, screw speed, torque and die pressure.

Since the FDA introduced its PAT initiative, several process analytical technology tools for pharmaceutical hot-melt extrusion processes were evaluated. Several research groups have proven that Raman spectroscopy is a valuable tool for in-line monitoring of API concentration and solid state during a pharmaceutical hot-melt extrusion process [29, 30]. Using on-line spectroscopic techniques, PAT can also lead to a better understanding of the HME process. Currently a lot of research on PAT in hot-melt extrusion is being performed. Terahertz pulsed imaging [31] seems a promising technique for analyzing the final co-extruded product in the future, since it not only characterizes the outer but also the inner layer of the co-extrudate.

MATERIALS USED IN CO-EXTRUSION

To be processed by HME, a material must deform in the extruder and solidify upon exiting the die. HME formulations for pharmaceutical dosage forms are mixtures of an API and functional excipients, mainly classified as matrix carriers, plasticizers and other additives (e.g. anti-oxidants and release modifying agents).

An appropriate carrier selection is important in the development of a hot-melt extruded dosage form. Carrier materials used can be polymeric materials - generally thermoplastic polymers exhibiting a low glass transition temperature (*Tg*) or melting point - or thermodeformable waxes. The properties of the carrier determine the processing conditions and drug release kinetics from the final drug product [1]. Besides the glass transition temperature, the melt viscosity determines the processing temperature of a polymer, since the torque produced within the extruder - which depends on the viscosity of the melt - determines whether extrusion is possible or not [4]. The main challenge for a co-extrusion process is to find appropriate polymer combinations, taking into account the pharmaceutical aspects (e.g. meeting the required drug release characteristics), regulatory requirements (e.g. fulfilling the Generally Recognized As Safe (GRAS) status) as well as some technical considerations (e.g. similar extrusion temperature at the die, melt viscosity matching, adhesion between layers).

The use of polymeric carriers may require the incorporation of a plasticizer - a low molecular weight compound able to decrease *Tg* and melt viscosity of a polymer by increasing the free volume between polymer chains - to improve extrusion conditions (lower temperature and less torque) or to improve the physical and mechanical properties of the final drug product. Citrate esters, fatty acid esters and sebacate esters, but also low molecular weight

polyethylene glycols and surfactants are commonly used as plasticizers in pharmaceutical dosage forms [1].

Additives can be added to the formulation for different reasons, e.g. the stability of polymers that are prone to degradation can be improved with the addition of anti-oxidants [32]. Other functional excipients can modify the drug release rate, e.g. viscosity increasing agents can be incorporated into a polymer matrix to reduce an initial burst release.

For each individual component, including the API, thermal stability is a prerequisite to be processed via HME. However, the short processing times using twin screw extruders may not limit all thermolabile compounds [1].

For the development of a co-extruded formulation some additional technical considerations have to be taken into account: (a) each melt needs to flow through the co-extrusion die under the same temperature conditions, (b) the melt viscosity of the different layers need to match to avoid layer non-uniformity [16], (c) an adequate adhesion between the layers is indispensable to avoid separation during downstream processing [33], (d) the degree of interdiffusion and migration between the co-extruded layers needs to be considered and (e) shrinkage of both layers should not differ too much [34]. Taking into account these extra considerations it is obvious that formulating the different layers in co-extrusion is not always straightforward.

MEDICAL AND PHARMACEUTICAL APPLICATIONS

In medical applications extrusion was already used for years to produce balloon tubing and single- or multi-lumen tubing, to be used for minimally invasive diagnostic and therapeutic procedures. Now the technology has advanced to co-extrusion in order to create tubing with multiple layers of different materials or with colored stripes of the same material. Co-extruded tubing is especially useful for angioplasty, placing stents, guiding catheters and dialysis [35]. For multiple layer tubings the different materials (e.g. silicone, nylon, polyurethanes) are selected to be compatible to prevent delamination of the tubing. Chemically dissimilar materials can be extruded together using a tie layer as an intermediate layer between the core layer and the outer layer. The co-extruded multilayer tubings show enhanced performance characteristics, since materials with different but complementing properties can be combined. A tri-layer tubing with a thin hydrophilic surface coating can e.g. provide low friction for the advancement of a guide wire or catheter through the lumen without comprising strength and stiffness. The stripes on a co-extruded tubing can be coloured, contain radiopaque materials that make the tubing visible on X-rays or ensure built-in lubricity. Co-extrusion is gaining importance in tubing for medical use with multiple layers and thinner walls. Therefore unprecedented levels of dimensional accuracy and flaw detection are required. As tubes get smaller, and walls and layers get thinner, medical tubing manufacturers have to put more emphasis on gauging for quality control [36].

In medical devices there is a trend to combination products where a medical device is loaded with a drug in order to deliver it at the site of action or to influence the release of the loaded drug. Some examples are drug-eluting coronary stents, contraceptive implants, vaginal rings and transdermal patches. The design of advanced drug-delivery systems has moved to the

forefront in pharmaceutical technology. In the design of such systems, co-extrusion and supporting downstream solutions can provide unique advantages.

The commercially available drug products that are produced by means of co-extrusion are in most cases combinations of a drug with a medical device, e.g. Implanon® and Nuvaring®. Implanon® (Schering-Plough), a non-biodegradable flexible rod that contains etonogestrel, provides contraceptive efficacy during a period of 3 years [37]. The rod is 4 cm in length by 2 mm in diameter and consists of a solid core of ethylene vinyl acetate (EVA) with embedded etonogestrel crystals. The core is surrounded by an outer ethylene vinyl acetate membrane that controls the release rate. The ends of the rod are not covered by an outer layer to allow an initial rapid hormone burst after the implant is inserted in the arm, just under the skin [38]. Nuvaring® (MSD), a contraceptive intravaginal ring releasing etonogestrel and ethinyl estradiol for 21 days, consists of a coaxial fiber, prepared by ethylene vinyl acetate copolymers. The coaxial fiber consists of a core polymer - with two steroids incorporated in a molecularly dissolved state - that is enveloped with a thin polymer membrane. The outer EVA membrane regulates drug release and provides a near zero-order rate of release over a 21-day period. This polymeric reservoir system is prepared by co-extrusion, consisting of two single screw extruders connected to a spinning block. The molten polymers for core and membrane are delivered to two gear pumps and subsequently combined in a spinneret, thereby forming the coaxial fiber [39]. Following extrusion, the rod is cut and end-fused to create a ring [40].

Some pharmaceutical products are composed of two extruded layers, but these were not extruded simultaneously. The Egalet® technology, an erosion-based delayed-release system, consists of an impermeable shell with a plug of active drug as the core of the drug delivery system [41]. The shell consists of polyethylene glycol monostearate combined with higher

molecular weight polyethylene glycols and polyethylene oxides. During the injection molding production process the shell is formed during the first injection in the mold and afterwards the core is molded during a second injection. This manufacturing process provides a high accuracy in the dimensions of the matrix, which is very important since the drug delivery system is designed to show a continuous zero-order release, directly proportional to the area eroded [42]. Jadelle, a non-biodegradable, flexible, subdermal levonorgestrel implant for contraception consists of two rods of dimethylsiloxane/methylvinylsiloxane copolymer. The core of each rod is a 1:1 physical mixture of silicone rubber elastomer and levonorgestrel, and is covered with a thin silicone rubber tubing. The rod is cured in an oven to cross-link tube and drug core. After the open ends of the tubing are sealed with medical adhesive the implants are sterilized [43].

Only recently co-extrusion is gaining importance in the production of oral drug products. So far, there are no co-extruded dosage forms for oral use on the market, but several research studies have been performed in this field. Quintavalle et al. characterized hot-melt co-extruded cylindrical systems for controlled drug delivery. The sustained release profile was obtained by extruding two concentric theophylline-loaded matrix formulations: an inner hydrophilic polyethylene glycol-based matrix combined with an outer lipophilic layer, mainly consisting of microcrystalline wax. A screening of devices differing in dimensions and relative proportions of inner and outer layer was performed based on *in vitro* drug release. The release mechanisms were studied using a mathematical model [7, 44]. Iosio et al. produced pellets with two cohesive layers via co-extrusion/spheronization. An inert layer of microcrystalline cellulose, lactose and water was combined with a second layer containing a self-emulsifying system of the model drug vinpocetine. In order to evaluate the effects of formulation variables an experimental design was used. *In vitro* dissolution and *in vivo* tests

demonstrated that it was possible to produce bilayered cohesive self-emulsifying pellets by means of co-extrusion/spheronization, with improved solubility and *in vivo* bioavailability of the poorly water-soluble model drug [22]. A recent study by Dierickx et al. described the successful preparation of fixed-dose combination mini-matrices via co-extrusion [11]. A core/coat dosage form was developed, wherein the core and coat exhibited sustained and immediate release properties respectively, using a combination of polycaprolactone (core) and polyethylene oxide/polyethylene glycol (coat). The same research group also developed by means of co-extrusion a multilayered dosage form characterized by a dual release profile of the same drug. The co-extrudates consisted of two concentric polymer matrices: a core having a lipophilic character, and a coat with a hydrophilic character. The maximum drug load in core and coat was determined by the extrusion temperature and the die dimensions, while adhesion between core and coat was mainly determined by the drug load and the extrusion temperature [45].

FIXED-DOSE COMBINATION PRODUCTS FOR COMBINATION THERAPY

The production of oral drug delivery systems via co-extrusion offers the opportunity to produce fixed-dose combination products. Combination therapy with two or more agents having complementary mechanisms of action can extend the range of therapeutic options in the treatment of numerous human diseases. Combination therapy is increasingly recognized as a major advantage, not only for life cycle management of drugs but also for therapeutic reasons. One major benefit of combination therapies is that they reduce development of drug resistance, since a pathogen or tumor is less likely to have resistance to multiple drugs simultaneously [46]. The combination of pharmaceutical products offers enhanced efficacy and improved selectivity - and therefore reduced toxicity and side effects - of the drugs [47, 48].

Fixed-dose combination (FDC) products contain two or more active pharmaceutical ingredients in a single dosage form. Oral combination drug delivery systems have proven to be highly beneficial in the treatment of life threatening diseases such as cancer, AIDS and tuberculosis. Additionally combination drug therapy has been successfully introduced for diseases such as diabetes, heart diseases, central nervous system disorders and to treat microbial infections [47]. They become increasingly important, given that 26 out of the 101 FDA-approved New Drug Applications (NDAs) in 2010 were FDC products [49]. The combination of two or more drugs with complementary modes of action optimizes treatment and offers convenience, reduced dosing unit burden and cost savings to the patient. For tuberculosis treatment the (WHO) recommends the use of a 4-drug FDC preventing patients to be selective in their choice of drugs and the resulting acquisition of drug resistance due to monotherapy [50]. Also for oral anti-diabetic therapy fixed-dose

combination dosage forms have shown to simplify pill regimen and improve patient adherence [51, 52]. These advantages are especially important for the aging population in developed countries needing multiple medications to treat age-related diseases and co-morbidities. In this respect the use of polypills - containing a statin, blood pressure-lowering agents and aspirin - and their impact on adherence was also positively evaluated [53]. Sometimes two drugs are also combined to minimize the potential for abuse of the principal active component, e.g. the addition of naloxone, an opioid antagonist, to buprenorphine to prevent the possibility of injecting the product by opioid addicts.

An important disadvantage of FDC products is the reduced dosing flexibility, which is often compensated by the development of multiple strength combinations. However, for treatments requiring frequent dose adjustments FDC products are not very useful. Another disadvantage might be that, since the FDC contains multiple drugs in one tablet, the tablet size may be too large to swallow for pediatric and elderly patients. Additionally it is also more difficult to determine which drug caused an adverse drug reaction [49]. Moreover, the goal of a combination therapy is additive benefit or preferentially synergy, but the substantial risks need to be recognized. Treatments that are safe when used alone can promote drug-drug interactions, which may induce changes in pharmacokinetics [54].

As a regulatory strategy for FDC products, establishing bioequivalence of drugs in an FDC product to drugs co-administered as individual entities is a very common approach. In addition possible food effects need to be considered as well [49]. Investigational drugs are traditionally tested for efficacy in add-on trials, in which the new drug added to a standard treatment is compared with the standard regimen alone. But, for diseases in which innovative targeted combination therapies are developed such clinical studies will often be unethical because of the potential to promote the development of resistance. Therefore FDA

has drafted a guidance for industry concerning the co-development of two or more unmarketed investigational drugs for use in combination, providing some flexibility in establishing the contribution of the individual drugs [55]. The additional uncertainty introduced by this co-development approach should not be underestimated, because it will not be possible to fully characterize the effects of the individual components of the combination. For this reason co-development should be used only for treatments of serious life-threatening diseases. FDA recommends adequate pharmacovigilance plans to address the risks linked to co-development [55].

Formulation and manufacturing of an FDC is a challenge and is critical to ensure unaffected bioavailability of each of the components. Incorporation of several drugs in one film-coated tablet might compromise bioavailability of one component, e.g. when a water-insoluble component is incorporated with highly water-soluble drugs [56]. Therefore several innovative drug delivery systems have been developed. Numerous bilayer tablets have shown their potential to incorporate two drugs in different layers and provide adequate drug release for each component, by e.g. compressing granules containing different drugs and excipients [57]. Multilayered tablets with a drug-loaded film coat surrounding a core containing the second drug in an appropriate matrix have been successfully developed and marketed [58]. Another multidrug and multikinetics delivery system developed is the Dome Matrix®, where tablets having a specific shape are assembled into a multimodule system [59]. Filling controlled- and immediate-release prills, loaded with different drugs, into a capsule also proved to be a successful method to develop a fixed-dose combination [60]. The commercially available PRODAS® technology is a multi-unit system combining the benefits of tableting technology within a capsule as a number of mini-tablets are combined in a hard gelatin capsule. Several innovative technologies even use capsule-in-capsule systems to offer

multiphase multicompartment combination product delivery systems (InnerCap™ and DuoCap™). However, as all of these delivery systems require a multistep production process, co-extrusion can be an attractive alternative.

DISCUSSION

Drug development was never more challenging as pharmaceutical production processes with a better efficiency must be developed, while drug substances are often more difficult to process because their often poor bioavailability. Hot-melt co-extrusion is the perfect answer to these challenges, as it provides the possibility to formulate a drug substance as a solid dispersion via a continuous process. Since combination products are gaining importance therapeutically and optimal drug delivery is a prerequisite for a new drug product, co-extrusion is a very promising formulation technique. When combining several drug substances in different matrices the release patterns of the drug delivery systems can be tailored to achieve the maximum therapeutic efficiency via a patient-friendly dosage form. Although optimization of downstream processing after co-extrusion remains a point of attention, there are several interesting options to obtain monolithic systems or multiparticulates, the latter having the advantage of dosing flexibility and small size, important for specific populations.

Barriers to the implementation of co-extrusion in the pharmaceutical industry are the process knowledge needed, the significant investment initially required and the limited number of established-use thermoplastic polymers available.

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CHAPTER 1

HOT-MELT CO-EXTRUSION FOR THE PRODUCTION OF FIXED-DOSE COMBINATION PRODUCTS WITH A CONTROLLED RELEASE ETHYLCELLULOSE MATRIX CORE

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ABSTRACT

Chapter 1 evaluates hot-melt co-extrusion as a technique for the production of fixed-dose combination products, using ethylcellulose as a core matrix former to control the release of metoprolol tartrate and a polyethylene oxide-based coat formulation to obtain immediate release of hydrochlorothiazide. By lowering the concentration of the hydrophilic additive polyethylene oxide in the plasticized ethylcellulose matrix or by lowering the drug load, the *in vitro* metoprolol tartrate release from the core was substantially sustained. The *in vitro* release of hydrochlorothiazide from the polyethylene oxide/polyethylene glycol coat was completed within 45 min for all formulations. Tensile testing of the core/coat mini-matrices revealed an adequate adhesion between the two layers. Raman mapping showed no migration of active substances. Physicochemical state characterization indicated that the crystalline state of metoprolol tartrate was not affected by thermal processing via hot-melt extrusion, while hydrochlorothiazide was dissolved in the coat. These physicochemical characteristics were confirmed during the stability study. Considering the bioavailability of metoprolol tartrate after oral administration to dogs, the different co-extruded formulations offered a range of sustained release characteristics. Moreover, high metoprolol tartrate plasma concentrations were reached in dogs allowing the administered dose to be halved. Interestingly, the resulting metoprolol tartrate plasma concentrations could be predicted based on the observed *in vitro* release kinetics, using an appropriate mathematical model. The latter takes into account drug transport within the dosage form as well as the *in vivo* fate of the drug.

CHAPTER 1

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INTRODUCTION

The need for novel combination therapies, primarily focusing on fixed-dose combinations (FDC), has been reported by various authors and is seen as a driver for innovative drug development [1, 2]. Besides their benefits in life cycle management, FDC products have shown to improve patient adherence by decreasing the number of required pills and thus reducing the complexity of the medication regimen [3]. Fixed-dose combinations offer benefits to a lot of drugs due to the additive nature of therapeutic effect and the reduced level of side-effects associated with the use of complementary drugs [4]. The application of oral sustained release formulations has improved patient compliance due to a lower dosing frequency and a reduced incidence of adverse side effects. Sustained release formulations have shown to offer many other advantages over conventional drug products, such as the

controlled administration of a therapeutic dose at a desired delivery rate in order to gain more constant plasma concentrations of the drug. Moreover, the production of sustained release multiparticulate dosage forms is advantageous since *in vivo* the subunits spread into the gastrointestinal tract as soon as the dosage unit, e.g. capsule or tablet, disintegrates. Since high local drug concentrations are avoided, less inter- and intra-subject variability and a decreased risk of dose dumping can be expected [5].

While hot-melt extrusion (HME) has proven to be a successful processing technique used in pharmaceutical industry to produce drug products in a continuous way, co-extrusion is quite new in pharmaceutical applications [6, 7]. Nevertheless co-extrusion of polymers is widely applied in the plastics and packaging industry. The pharmaceutical co-extrusion process consists of the simultaneous hot-melt extrusion of two or more drug-loaded formulations creating a multilayered extrudate. HME as a continuous manufacturing technology has shown some other major advantages over conventional techniques, like improving the bioavailability of poorly water-soluble drugs via molecular dispersions [8], without the requirement for processes based on organic solvent or aqueous spray drying. Moreover via HME matrix formulations can be manufactured using polymers that act as drug depots [9]. The added value of co-extrusion is that it allows to modulate the release of each drug independently, to enable simultaneous administration of non-compatible drugs and to produce fixed-dose combinations in a continuous single-step process. By processing the co-extrudate into mini-matrices that can be easily filled into gelatin capsules a multiparticulate formulation is created. A specific challenge during co-extrusion is to establish a core/coat polymer combination fit for purpose considering required release characteristics of the incorporated drugs, similarity in extrusion temperature and appropriate adhesion between the layers. So far, no co-extruded dosage forms for oral use are on the market. In this study a

contribution is made to enable the use of co-extrusion in pharmaceutical industry for the production of oral FDC drug products that offer multiple release profiles.

The aim of this study was to evaluate the use of co-extrusion for the manufacturing of a fixed-dose combination drug product for oral application, using a core matrix former that offers a range of controlled release profiles for highly water-soluble drugs. For this purpose ethylcellulose, a thermoplastic polymer that has been intensively used as a matrix former in hot-melt extrusion [10, 11], was combined with polyethylene oxide as a hydrophilic additive and metoprolol tartrate as model drug. The combination of this beta-blocker with the diuretic hydrochlorothiazide is well known [12]. It offers the opportunity for a co-extrudate with hydrochlorothiazide incorporated in the coat as immediate release model drug and metoprolol tartrate incorporated in the core as model for a highly water-soluble drug. The *in vitro* performance of the different formulations was assessed. The physicochemical state of the model drugs in the formulations was characterized using modulated differential scanning calorimetry (MDSC), X-ray diffraction (XRD) and Raman spectroscopy. Furthermore, the physical stability of the co-extruded mini-matrices was monitored during 6 months storage at 25 °C/60 %RH and 40 °C/75 %RH. Finally, the bioavailability of the different formulations was evaluated after oral administration to dogs and compared to a commercially available fixed-dose combination product. In addition, a mathematical model considering the controlled release of metoprolol tartrate from the dosage form and its fate *in vivo* was developed and used to predict the resulting drug plasma profiles, based on the *in vitro* results and *in vivo* parameters reported in literature.

MATERIALS AND METHODS

Materials

Metoprolol tartrate (Esteve Quimica, Barcelona, Spain) and hydrochlorothiazide (Utag, Amsterdam, the Netherlands) were selected as sustained release and immediate release model drugs, respectively. Ethylcellulose (Ethocel® std 10, Colorcon, Dartford Kent, United Kingdom), dibutyl sebacate (Sigma-Aldrich, Bornem, Belgium), polyethylene oxide 1M (MW: 1000000 g/mol, SentryTM Polyox® WSR N12K, Colorcon, Dartford Kent, United Kingdom), polyethylene oxide 100K (MW: 100000 g/mol, SentryTM Polyox® WSR N10, Colorcon, Dartford Kent, United Kingdom), polyethylene glycol 4K (MW: 4000 g/mol, Fagron, Waregem, Belgium), polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®, BASF, Ludwigshafen, Germany), poloxamer 188 (Lutrol F68®, BASF, Ludwigshafen, Germany) and an 8:2 blend of polyvinyl acetate and polyvinylpyrrolidone (Kollidon SR®, BASF, Ludwigshafen, Germany) were used as excipients. All other chemicals were of analytical grade.

Hot-melt co-extrusion

Co-extrusion was carried out with two co-rotating, fully intermeshing, Prism Eurolab 16mm twin screw extruders (ThermoFisher Scientific, Karlsruhe, Germany), both connected to a co-extrusion die (Guill, West Warwick, USA). The co-extrusion die combined both layers into a rod-like co-extrudate consisting of a core and a concentric coat. The heating segments of both extruders were heated to 80/90/100/100/100/100 °C from feed opening to die-end. The co-extrusion die was heated to 100 °C. Both formulated premixes were fed separately

into the corresponding extruder by a Brabender Flexwall® loss-in-weight powder feeder (Duisburg, Germany) at a feed rate of 200 g/h for the coat and 300 g/h for the core material. A screw speed of 40 rpm for the extruder producing the outer layer and 150 rpm for the extruder producing the inner layer was used. The core of the co-extrudate, with a diameter of 3 mm, was surrounded by a coat with a thickness of 0.5 mm, which led to a total co-extrudate diameter of 4 mm. Four different co-extrudates were manufactured consisting of a specific core and coat formulation, by combining the following components in different concentrations: ethylcellulose (EC), dibutyl sebacate (DBS), polyethylene oxide (PEO), polyethylene glycol (PEG), metoprolol tartrate (MPT) and hydrochlorothiazide (HCT) (Table 1). After cooling down the co-extruded rod to room temperature, the cylindrical co-extrudate was manually cut into mini-matrices of 2 mm length. Those mini-matrices had an average weight of 27.2 mg (SD = 1.8 mg, n = 20).

		Matrix			Drug load	Drug dose
Formulation A	Core	53% EC	27% DBS	20% PEO1M	30% MPT	200 mg
	Coat	85% PEO100K		15% PEG 4K	5.6% HCT	25 mg
Formulation B	Core	62% EC	33% DBS	5% PEO 1M	30% MPT	200 mg
	Coat	85% PEO100K		15% PEG 4K	5.6% HCT	25 mg
Formulation C	Core	53% EC	27% DBS	20% PEO1M	15% MPT	200 mg
	Coat	85% PEO100K		15% PEG 4K	2.8% HCT	25 mg
Formulation D	Core	62% EC	33% DBS	5% PEO 1M	30% MPT	100 mg
	Coat	85% PEO100K		15% PEG 4K	11.2% HCT	25 mg
Reference	Zok-Zid® (Pfizer, Brussels, Belgium)				MPT*	200 mg
					HCT	25 mg

* Original reference product contained metoprolol succinate; dose calculated as metoprolol tartrate.

Table 1. Composition of test and reference formulations and drug dose administered during the *in vivo* study. Formulation components are ethylcellulose (EC), dibutyl sebacate (DBS), polyethylene oxide (PEO), polyethylene glycol (PEG), metoprolol tartrate (MPT) and hydrochlorothiazide (HCT).

In vitro drug release

In vitro dissolution was performed using United States Pharmacopeia (USP) dissolution apparatus 1 (baskets). The equipment consisted of an Evolution 6300 dissolution system (Distek, New Brunswick, New Jersey, USA) coupled with an Evolution 4300 automatic dissolution sampler (Distek, New Brunswick, New Jersey, USA). The temperature of the dissolution medium (900 ml) was maintained at 37 ± 0.5 °C while the rotational speed of the baskets was set at 100 rpm. For the first hour a 0.1 N solution of hydrochloric acid (pH 1) was used as dissolution medium in order to mimic the pH of the stomach. Afterwards the baskets containing the mini-matrices were transferred to vessels filled with phosphate buffer pH 6.8 (USP) as a dissolution medium, to which they were exposed for the next 23 hours. Samples (filtered using Distek 45 µm filters) of 5 ml were withdrawn at 5, 10, 15, 20, 30, 45 and 60 minutes for the determination of hydrochlorothiazide in the first dissolution medium and at 1, 2, 4, 6, 8, 12, 16, 20 and 24 h for the determination of metoprolol tartrate in the second dissolution medium. The inner layer extrudate was analyzed separately to cover for the metoprolol tartrate release during the first hour. Samples were analyzed spectrophotometrically at 316.6 nm and 222 nm respectively, by an ultraviolet (UV) - spectrophotometer, type UV-1800 (Shimadzu, Deurne, Belgium), using an appropriate calibration curve for quantification of hydrochlorothiazide and metoprolol tartrate, respectively. Each experiment was performed in triplicate.

Modulated differential scanning calorimetry

The crystallinity of the drug in the matrices and the thermal behavior of pure compounds, physical mixtures and corresponding extrudates were studied using a differential scanning

calorimeter Q2000 V24.8 equipped with a refrigerated cooling system (TA Instruments, Leatherhead, UK). Nitrogen was used as a purge gas through the DSC cell (50 ml/min) and the refrigerated cooling system (RCS) unit (300 ml/min). Samples (± 5 mg) were run in hermetically closed Tzero pans with perforated lid, supplied by TA Instruments, with an underlying heating rate of 2 °C/min. The modulation period and amplitude were set at 60 s and 0.318 °C, respectively (heat-iso method). Mass of sample pan and empty reference pan were taken into account. Temperature and enthalpy calibration was performed with an indium standard, whereas calibration of the heat capacity was performed using a sapphire standard. MDSC data were analyzed using the TA instruments Universal Analysis 2000 V4.7A software. Melting enthalpies were determined in the total heat flow signal. Melting temperatures were reported as peak temperatures. The glass transition temperature corresponds to the temperature at the midpoint of the heat capacity change (or Cp jump).

X-ray diffraction

Crystallinity was analyzed using X-ray diffraction (XRD) on pure compounds, physical mixtures and corresponding extrudates. X-ray diffraction was performed on a D5000 diffractometer with Cu Kα radiation (λ = 1.54 Å) (Siemens, Karlsruhe, Germany) and a voltage of 40 mV in the angular range (2θ) varying from 10 to 60° using a step scan mode with a step size of 0.02° and a measuring time of 1 s/step.

Adhesion

The adhesion between core and coat was analyzed using a tensile tester with a load cell capacity of 100 N (LF Plus, Lloyd Instruments, West Sussex, UK). The co-extruded mini-

matrices were placed on a metal disk with a central opening of 3.3 mm, above which the core of the co-extruded mini-matrices was positioned to make sure only the coat was supported by the device. A probe with a diameter of 2 mm was used to apply a downward force on the core (preload 1 N; extension rate 100 mm/min) and the maximum force needed to separate coat from core was measured. The test was repeated 10 times for each tested formulation.

Raman spectroscopy

The distribution of the different components in the coat and core of the co-extrudates was evaluated with Raman microscopic mapping using a Raman Rxn1 Microprobe (Kaiser Optical Systems Inc, Ann Arbor, MI, USA), equipped with an air-cooled charge coupled device (CCD) detector. The laser wavelength employed was 785 nm from a Invictus near infrared (NIR) diode laser (Kaiser Optical Systems Inc, Ann Arbor, MI, USA). All spectra were recorded with a resolution of 4 cm^{-1} and an exposure time of 2 s, using a laser power of 400 mW. Cross sections of co-extrudates were scanned by a 10 x long working distance objective lens in point-by-point mapping mode using a step size of 50 µm in both the x and y directions (18 x 13 = 234 spectra per mapping). The resulting images provide information about the distribution of different components in the co-extrudates. Data collection and data transfer were automated using the HoloGRAMS™ data collection software (version 2.3.5, Kaiser Optical Systems Inc, Ann Arbor, MI, USA), the HoloMAP™ data analysis software (version 2.3.5, Kaiser Optical Systems Inc, Ann Arbor, MI, USA) and Matlab® software (version 7.1, The MathWorks Inc., Natick, MA, USA). In order to correct for the uneven surface of the co-extrudates, manually cut in half using a surgical blade, all spectra were preprocessed using Pearsons method to perform a baseline correction.

In order to attribute specific Raman peaks in the spectra to the different components in the formulations, Raman spectra were collected from the pure components and the physical mixtures. All spectra were recorded with a resolution of 4 cm^{-1} and an exposure time of 5 s. Standard normal variate (SNV) pre-processing was applied on the collected spectra prior to analysis, to correct for the variation in path length/sampling distance between probe and sample.

Stability study

A sufficient number of mini-matrices from the 3 different formulations was manufactured to perform a stability study. Immediately after co-extrusion, the formulations were filled in an amber glass container and stored in closed condition at 25 °C/60 %RH and in open condition at 40 °C/75 %RH. To investigate the influence of storage MDSC, XRD, and *in vitro* drug release were performed on the co-extrudates immediately after manufacturing, after 1 month, 3 months and 6 months storage, respectively.

In vivo evaluation

Study design

All procedures were performed after approval by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (Merelbeke, Belgium). To study the drug plasma profiles for hydrochlorothiazide and metoprolol tartrate, the formulations listed in Table 1 were administered to 6 male mixed-breed dogs (23 - 41.5 kg). For the test formulations A, B, C and D the co-extruded mini-matrices were filled in hard-gelatin capsules, whereas the reference formulation was given as 2 tablets Zok-Zid® (Pfizer, Brussels, Belgium). During the

cross-over study the formulations were administered in randomized order, taking into account a wash-out period of at least 8 days. The dogs were fasted for 12 h prior to and after the administration of the formulations, but water was available ad libitum. At the start of the study an intravenous cannula was placed in the lateral saphenous and a blank blood sample was collected. The formulations were orally administered with 20 ml of water and blood samples were subsequently collected in dry heparinized tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after administration. The blood samples obtained were centrifuged for 5 min at 1500 g within 1 h after collection. The plasma was separated and frozen at -20 °C before analysis.

Metoprolol tartrate and hydrochlorothiazide assay

Two different validated high performance liquid chromatography (HPLC) methods were used to determine metoprolol tartrate and hydrochlorothiazide plasma concentrations in the dog plasma. After extraction of metoprolol tartrate and the internal standard bisoprolol hemifumarate from the plasma using a solid phase extraction (SPE) procedure, with Oasis® MCX 1 cc (30 mg) cartridges (Waters, Brussels, Belgium), a HPLC method with fluorescence detection was used for the determination of metoprolol tartrate [13]. Hydrochlorothiazide was extracted from the plasma samples by means of a liquid-liquid extraction with the internal standard hydroflumethiazide and afterwards determined using a HPLC-UV method [14]. The HPLC system consisted of an isocratic solvent pump (L-7100, Merck, Hitachi LaChrom, Tokyo, Japan) and an automatic autosampler (L-2200, Merck, Hitachi Elite LaChrom, Tokyo, Japan). For the MPT analysis a guard column (LiChroCart® 4-4, LiChrospher® 100 CN (5 µm), Merck, Darmstadt, Germany) followed by a reversed phase CN column (LiChroCart® 250-4, LiChrospher® 100 CN (5 µm), Merck, Darmstadt, Germany) and a

variable wavelength fluorescence detector (L-7480, Merck, Hitachi LaChrom, Tokyo, Japan) were used. The pump flow was set at 1.1 ml/min and the excitation and emission wavelength were 275 nm and 300 nm, respectively. For the HCT analysis a guard column (LiChroCart® 4-4, LiChrospher® 100 RP-18e (5 µm), Merck, Darmstadt, Germany) followed by a reversed phase column (LiChroCart® 250-4, LiChrospher® 100 RP-18 (5 µm), Merck, Darmstadt, Germany) and a UV-detector (L-7400, Merck, Hitachi LaChrom, Tokyo, Japan) were used. The pump flow was set at 0.8 ml/min and the detection wavelength was 272 nm. Since metoprolol tartrate did not cause an interfering peak during the determination of hydrochlorothiazide, and vice versa, the specificity of the methods was secured. Peak integration was performed using the software package D-7000 HSM Chromatography Data Station (Hitachi Instruments, San Jose, CA, USA).

Data analysis

The metoprolol tartrate and hydrochlorothiazide plasma concentrations were normalized for dose and body weight of the dogs, by dividing the respective plasma concentrations by dose per kg body weight for each dog. The peak plasma concentration (C_{max}), the extent of absorption ($AUC_{0\text{-}12h}$) and the time to reach the peak plasma concentration (t_{max}) were calculated. The controlled release characteristics of the core formulation were evaluated by means of the $HVD_{t50\%Cmax}$, defined as the width of the plasma concentration profile at 50% of the C_{max} [15]. The bioavailability data were statistically evaluated using SPSS 17 (SPSS, Chicago, USA). To compare the effects of the different treatments on the pharmacokinetic parameters, a multiple comparison among pairs of means was performed using a Bonferroni post-hoc test with $p < 0.05$ as significance level.

RESULTS AND DISCUSSION

Formulation and production of co-extrudates

In order to produce a combination product with an immediate release coat and a controlled release core via co-extrusion some combinations of polymer-plasticizer mixtures were hot-melt extruded and evaluated for their processability. A successful co-extrusion process requires that both polymer melts can be processed at similar temperatures, match in melt viscosity and show adequate adhesion. Therefore combinations of polymer-plasticizer mixtures for core and coat were tested. As a first combination Kollidon SR® was assessed for the controlled release core and Soluplus® for the immediate release coat. At a metoprolol tartrate load of 30 %, Kollidon SR® only slightly sustained the release (80 and 100 % released at 2 and 8 h, respectively). The addition of the hydrophobic plasticizer dibutyl sebacate (15 %) only minimally improved the sustained release profile (with 80 and 100 % released at 4 and 8 h, respectively). Soluplus® was initially assessed in combination with a wide range of plasticizers. The lowest possible extrusion temperature (130 °C) was reached for the formulation containing 10 % Lutrol F68®. This coat formulation only offered an immediate release profile for hydrochlorothiazide concentrations of 5 % or less. A second core/coat combination tested consisted of an ethylcellulose matrix for the controlled release core and a polyethylene oxide 100K/polyethylene glycol 4K immediate release coat. Dibutyl sebacate has previously been used to plasticize an ethylcellulose matrix and polyethylene glycol/polyethylene oxide was added in different concentrations to modify the metoprolol tartrate release [11, 16]. As a core matrix ethylcellulose, plasticized with dibutyl sebacate, sustained metoprolol tartrate release in an efficient way, but by adding a hydrophilic additive to this matrix it became possible to offer a range of release profiles for metoprolol

tartrate release. Polyethylene oxide 1M was selected as a hydrophilic additive and a design of experiments (DOE) methodology was employed to set up 11 experiments (8 experiments + 3 center points) in a mixture design in order to extrude metoprolol tartrate at a drug load of 30 % in different combinations of ethylcellulose, dibutyl sebacate and polyethylene oxide 1M as a matrix. Two formulations with a similar ethylcellulose/dibutyl sebacate ratio but a major difference in metoprolol tartrate release were finally selected. Both had a drug load of 30 % metoprolol tartrate but a different matrix composition: (A) 53 % ethylcellulose + 27 % dibutyl sebacate + 20 % polyethylene oxide 1M and (B) 62 % ethylcellulose + 33 % dibutyl sebacate + 5 % polyethylene oxide 1M. In order to steer the metoprolol tartrate release not only by the amount of hydrophilic polymer (polyethylene oxide 1M) but also by drug load, this parameter was evaluated as a release-controlling factor as well. Therefore, a third formulation (C) was manufactured, containing 53 % ethylcellulose + 27 % dibutyl sebacate + 20 % polyethylene oxide 1M with a drug load of 15 % metoprolol tartrate in the core. For the corresponding coat formulation polyethylene oxide 100K, which has previously been used as a carrier in hot-melt extrusion, was chosen as main component [17, 18]. To optimize this coat formulation different concentrations of polyethylene glycol 4K were added to polyethylene oxide 100K and the hydrochlorothiazide release was evaluated [6]. A concentration of 15 % polyethylene glycol 4K proved to be sufficient for immediate hydrochlorothiazide release and consistently yielded co-extrudates of good quality with a smooth surface and good adhesion between core and coat (Fig. 1).

Figure 1. Image of co-extrudate, before and after cutting into mini-matrices of 2 mm length. Core diameter 3 mm and coat thickness 0.5 mm.

Therefore 85 % polyethylene oxide 100K + 15 % polyethylene glycol 4K was selected as matrix for the coat with the drug load adjusted in order to obtain a final formulation with the same metoprolol tartrate : hydrochlorothiazide ratio as the reference formulation (8:1). The metoprolol tartrate-loaded plasticized ethylcellulose matrix, with the addition of polyethylene oxide 1M as a hydrophilic additive, was finally co-extruded with the hydrochlorothiazide-loaded polyethylene oxide 100K/polyethylene glycol 4K coat at a temperature of 100 °C. The obtained co-extrudates had a regular shape, a smooth surface, a white opaque inner layer and a transparent outer layer.

In vitro drug release

The influence of both the hydrophilic additive and the drug load on metoprolol tartrate release from the core is illustrated in Fig. 2.

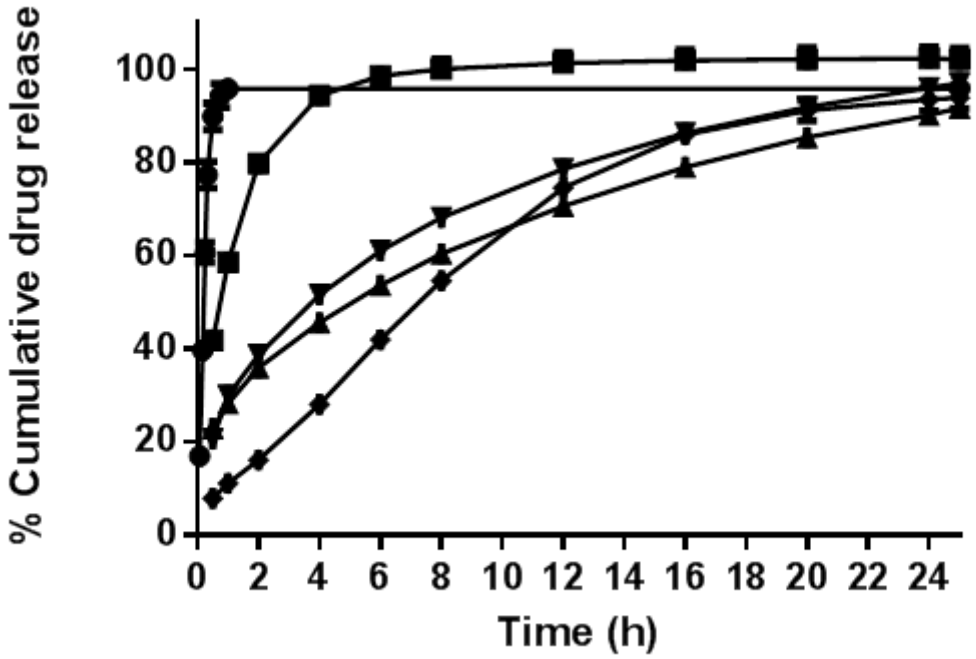

Figure 2. *In vitro* metoprolol tartrate release (in phosphate buffer pH 6.8) for formulation A ( ), formulation B ( ), formulation C ( ), reference ( ) and mean hydrochlorothiazide release (in 0.1 N HCl) for co-extrudate A to C ( ). Mean (n = 3) dissolution profiles (± SD) of each co-extrudate and reference. Dissolution at 37 °C and 100 rpm.

As previously reported the metoprolol tartrate release from the ethylcellulose matrix containing 20 % of polyethylene oxide 1M is diffusion controlled with a constant diffusion coefficient [19]. By reducing the amount of polyethylene oxide 1M in the matrix from 20 (formulation A) to 5 % (formulation B) the metoprolol tartrate release was found to be delayed from 80 and 100 % after 2 and 8 h, respectively, in formulation A to 36, 60, 70 and 90 % after 2, 8, 12 and 24 h, respectively, in formulation B. At this low polyethylene oxide 1M concentration it has been described that the mobility of the high molecular weight polyethylene oxide is increased with increasing matrix porosity over time, leading to an metoprolol tartrate release approximating a zero-order release [19]. Reducing the polyethylene oxide concentration lowered the burst release to around 20 % for formulation

B. The same delaying effect on the *in vitro* metoprolol tartrate release profile was seen by lowering the drug load from 30 % (formulation A) to 15 % (formulation C). The reference formulation, where metoprolol tartrate is formulated in coated pellets which are compressed into tablets, has a slightly different release profile for metoprolol tartrate compared to the matrix formulations B and C, showing a slower release during the first 8 hours and a lower burst release (8 % compared to 23 and 21 % for the matrix formulations B and C, respectively). For hydrochlorothiazide the immediate release criteria are met, with complete release obtained after 30 min for all formulations.

Physical state characterization

Fig. 3.a shows the MDSC thermograms of the core components, metoprolol tartrate, ethylcellulose : dibutyl sebacate physical mixture in a 2:1 ratio, polyethylene oxide 1M, the physical mixtures of a core formulation and the corresponding extruded formulation. Metoprolol tartrate showed a peak melting endotherm at 123.9 °C, while polyethylene oxide 1M showed a peak melting temperature at 70.2 °C, indicating the crystalline state of these compounds. The reversing heat flow for the 2:1 ethylcellulose : dibutyl sebacate mixture showed a clear change in heat capacity in the temperature range from 34 to 48 °C with a glass transition (*Tg*) at 39.5 °C. The indication that the 2:1 ethylcellulose : dibutyl sebacate mixture is in an amorphous state was confirmed by XRD analysis. Thermal analysis of the physical mixture and the extruded core formulation revealed that metoprolol tartrate remained crystalline after hot-melt extrusion. Fig. 3.b shows the MDSC thermograms for the components of the coat, hydrochlorothiazide, the 85:15 polyethylene oxide 100K : polyethylene glycol 4K matrix, the physical mixture and the corresponding extrudate.

Hydrochlorothiazide showed a peak melting temperature at 267.2 °C, while the polyethylene oxide 100K/polyethylene glycol 4K matrix showed a peak endotherm at 66.2 °C, indicating the crystalline state of these compounds. Thermal analysis of the physical mixture and the extruded coat formulation revealed only a melting endotherm of the polyethylene oxide 100K : polyethylene glycol 4K 85:15 matrix, indicating that hydrochlorothiazide is dissolved in the coat matrix. For the physical mixture this result is attributable to the heating of the sample during the MDSC experiment, where the small amount of hydrochlorothiazide in the physical mixture gradually dissolved in the molten polymer mixture during heating.

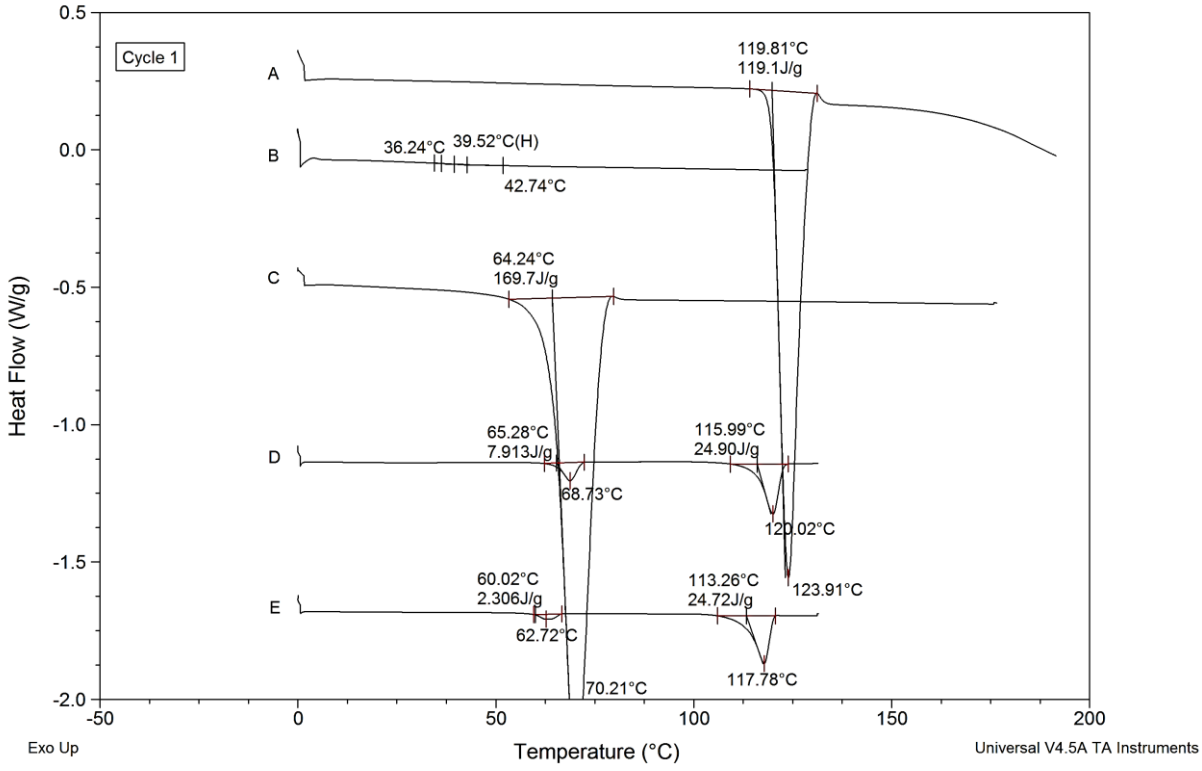

Figure 3.a. MDSC thermograms of metoprolol tartrate (A), ethylcellulose : dibutyl sebacate 2:1 (B), polyethylene oxide 1M (C), physical mixture of 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M with 30% metoprolol tartrate (D), extruded core formulation B: 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M with 30% metoprolol tartrate (E).

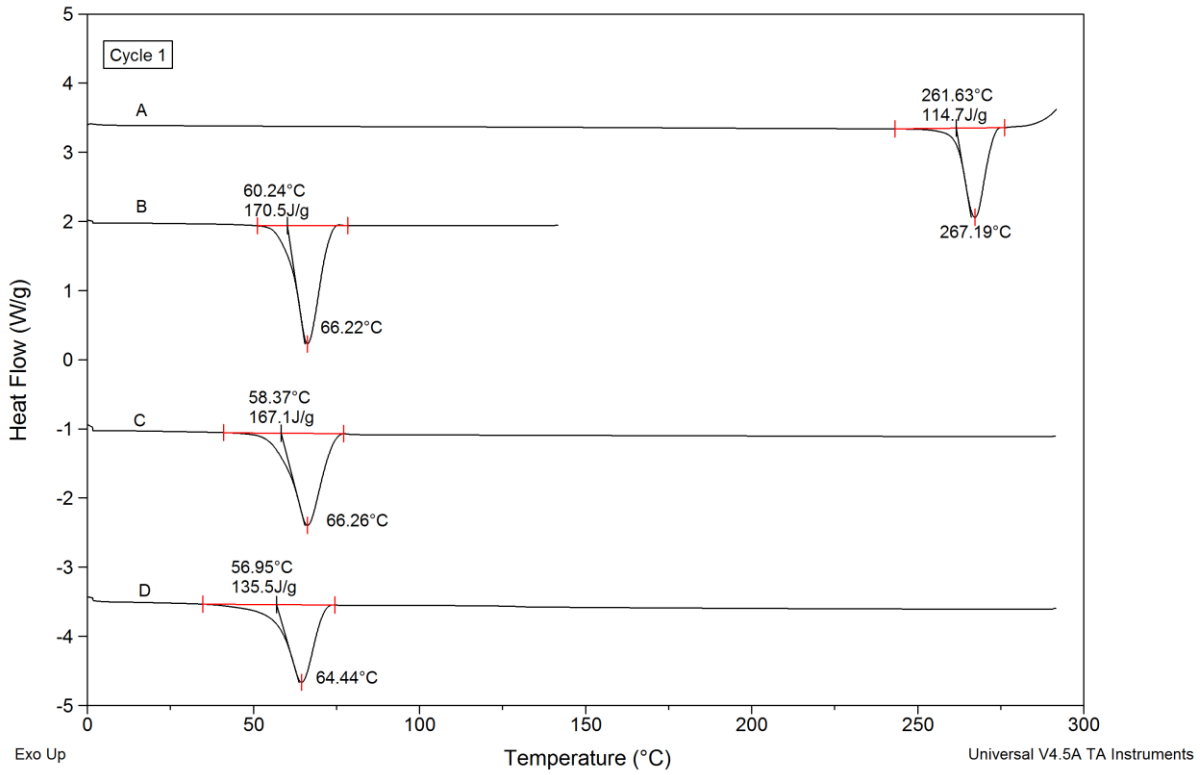

Figure 3.b. MDSC thermograms of hydrochlorothiazide (A), physical mixture of 85% polyethylene oxide 100K + 15% polyethylene glycol 4K (B), physical mixture of 85% polyethylene oxide 100K + 15% polyethylene glycol 4K with 5.6% hydrochlorothiazide (C), extruded coat formulation B: 85% polyethylene oxide 100K + 15% polyethylene glycol 4K with 5.6% hydrochlorothiazide (D).

The physicochemical state was also characterized using XRD and Raman spectroscopy. The X-ray diffraction patterns of the pure drug substances, the core and coat physical mixtures and the corresponding formulations are shown in Fig. 4. The X-ray diffractogram of metoprolol tartrate showed distinct diffraction peaks at 2θ of 10.7°, 16.0°, 19.6°, 20.5° and 23.3°. Since these peaks were also detected in the diffractogram of the physical mixture and the extruded core formulation, it can be concluded that the crystalline state of metoprolol tartrate was maintained in the hot-melt extrudates. The diffraction pattern of pure hydrochlorothiazide revealed several representative peaks, which also showed up in the diffractogram of the physical mixture of the coat. The absence of these peaks in the diffraction pattern of the extruded coat revealed that there were no hydrochlorothiazide

crystals left in the coat of the co-extrudate, confirming that hydrochlorothiazide was dissolved in the polyethylene oxide 100K : polyethylene glycol 4K 85:15 matrix after hot-melt extrusion.

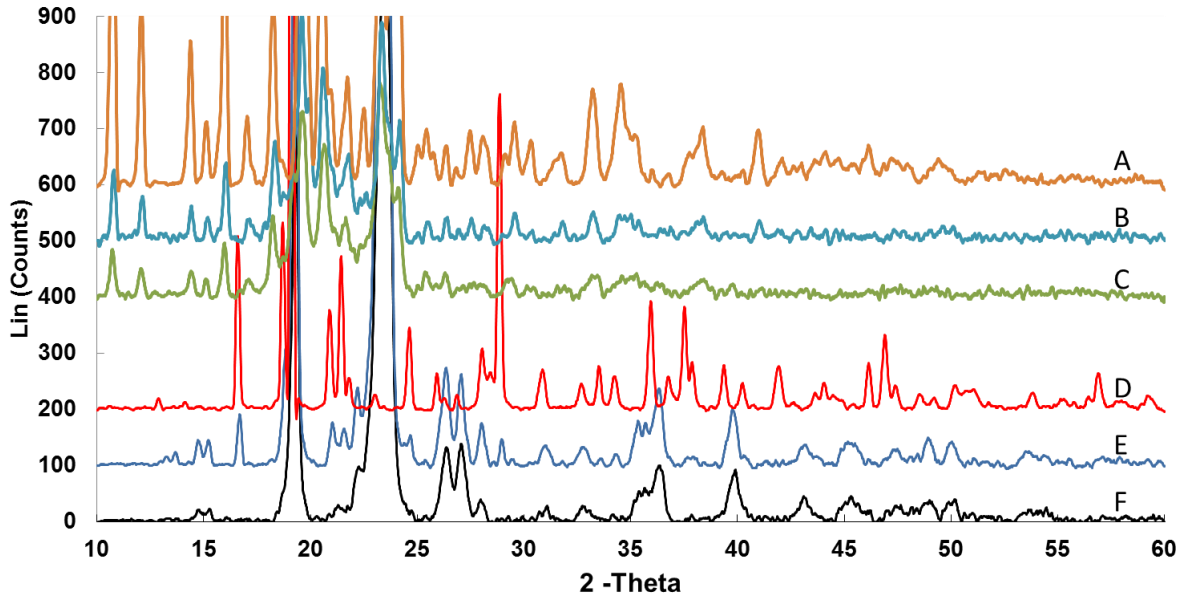

Figure 4. X-ray diffraction patterns of metoprolol tartrate (A), physical mixture core (B), co-extrudate core (C), hydrochlorothiazide (D), physical mixture coat (E), co-extrudate coat (F) for formulation A.

These results were confirmed with Raman spectroscopy. Raman peaks attributed to metoprolol tartrate, detected in the core of the co-extrudate, remained as sharp as in the physical mixture, indicating that metoprolol tartrate stayed in its crystalline state and the extrusion process did not affect the solid state of the metoprolol tartrate (Fig. 5).

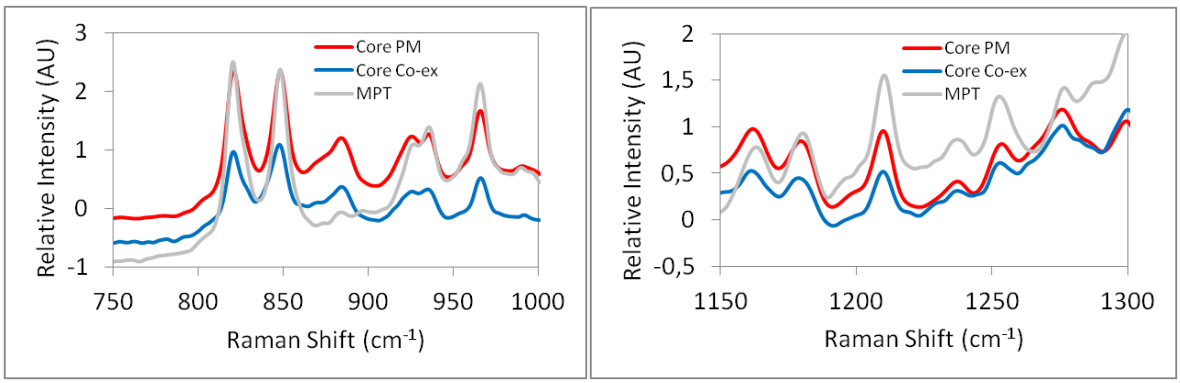

Figure 5. Selected regions of the Raman spectra of metoprolol tartrate, physical mixture of core and core of the co-extrudate for formulation B.

From the selected region of the Raman spectra in Fig. 6 it can be concluded that the Raman peaks attributed to hydrochlorothiazide in the coat of the co-extrudate showed broadening and a lower intensity when compared to the physical mixture, indicating the loss of crystallinity for hydrochlorothiazide.

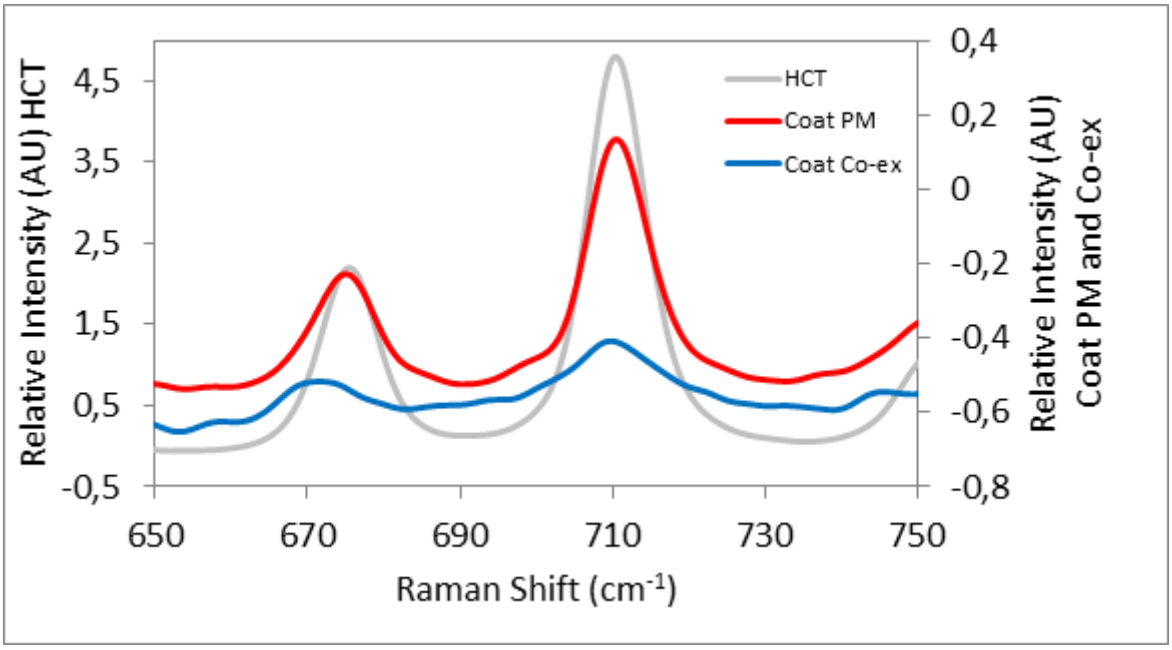

Figure 6. Selected region of the Raman spectra of hydrochlorothiazide, physical mixture of the coat and coat of the co-extrudate for formulation B. (Left scale for the selected region of the pure HCT spectrum. Right scale for the selected region of the spectra for physical mixture of the coat and co-extrudate of the coat.)

Co-extrudate characterization

The microscopic image of the co-extrudate clearly showed the two distinct layers, properly attached to each other. An adhesion test was performed in order to measure the adhesion force between core and coat. For the formulation with ethylcellulose 62 % + dibutyl sebacate 33 % + polyethylene oxide 5 % as a core matrix (formulation B), loaded with 30 % metoprolol tartrate the average force needed to detach the core from the coat was 8.3 ± 2.6 N. The

mini-matrices also passed a friability test (< 0.1 % weight loss), without any signs of detachment between core and coat layers.

To evaluate the distribution of the drug products in core and coat, Raman microscopic mapping was performed. The peak intensity of the Raman band of metoprolol tartrate in the 810 - 830 cm^{-1} region was monitored to map the distribution of metoprolol tartrate in the core and to check if there was diffusion of metoprolol tartrate in the coat. Fig. 7 shows the distribution of metoprolol tartrate, throughout different sections of the co-extruded core/coat formulation. A red color corresponds to a high peak intensity, indicating a high metoprolol tartrate concentration, while a blue color corresponds to a low metoprolol tartrate concentration. The metoprolol tartrate band in the 810 - 830 cm^{-1} region showed an intense peak in the core but not in the coat. The peak intensity of the Raman band of hydrochlorothiazide in the 700 - 720 cm^{-1} region was monitored to map the distribution of hydrochlorothiazide in the coat and to check if diffusion of hydrochlorothiazide in the core of the co-extrudate had occurred during hot-melt extrusion. The hydrochlorothiazide band in the 700 - 720 cm^{-1} region showed an intense peak in the coat but not in the core, indicating no intermigration of core and coat drug components during processing, given the spatial resolution of 50 µm.

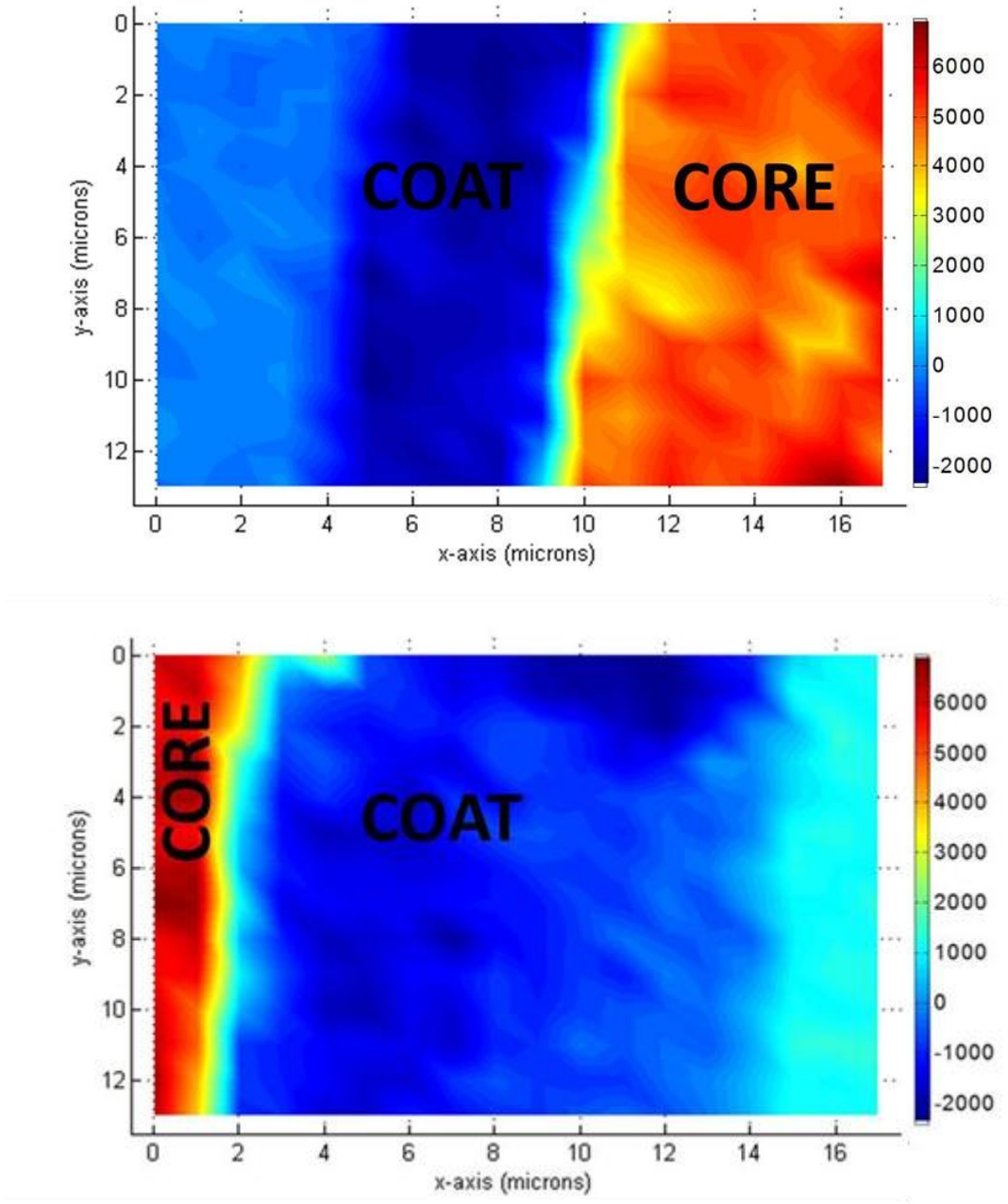

Figure 7. Raman mapping of metoprolol tartrate in two sections of the co-extrudate containing 30% metoprolol tartrate in the core. A red color corresponds to a high peak intensity in the 810 - 830 cm^{-1} region, indicating the presence of metoprolol tartrate. A blue color corresponds to a very low peak intensity, indicating absence of metoprolol tartrate.

Physical stability

Stability issues when using polyethylene oxide as a main matrix former in hot-melt extrusion have been reported previously. Polymer degradation of polyethylene oxide when stored

below its melting point was occurring more rapidly for the lower molecular weight polymer in comparison to the higher molecular weight polyethylene oxide and is due to oxygen permeation in the amorphous region of the semi-crystalline polymer [17]. Therefore, influences of storage conditions on drug release were investigated for the co-extruded formulations.

The co-extrudates of the three different formulations (A, B and C) kept their original shape and integrity during the entire stability study. The influence of storage on metoprolol tartrate release for formulation B is shown in Fig. 8. The same observations were made for hydrochlorothiazide release during the stability study. For both drugs the overall release profile remains similar over time and in both storage conditions.

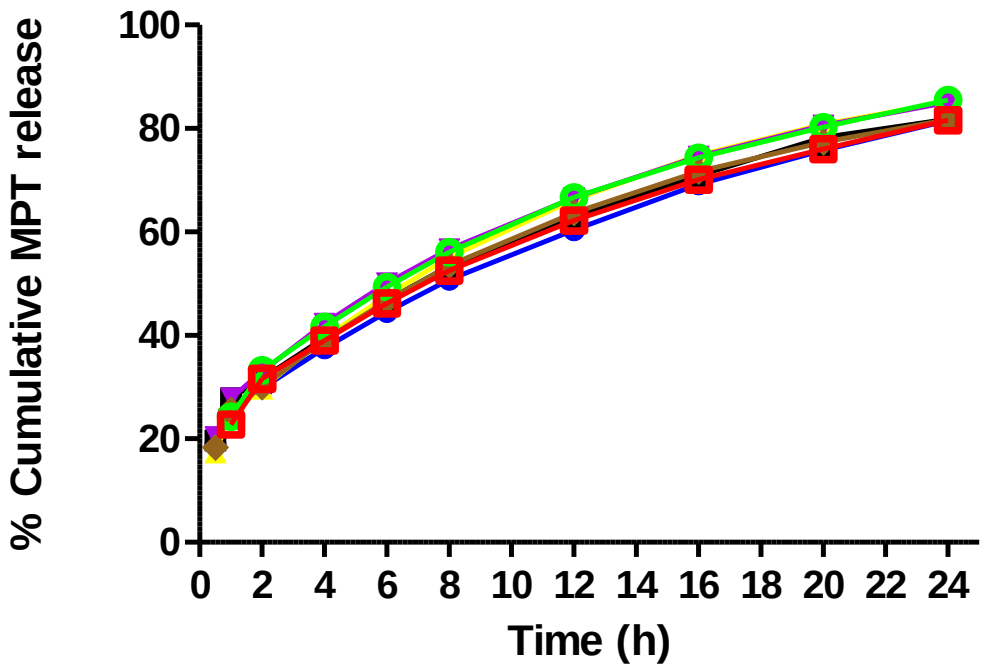

Figure 8. *In vitro* metoprolol tartrate (MPT) release (in phosphate buffer pH 6.8) for co-extrudate formulation B (Core: 62% ethylcellulose + 33% dibutyl sebacate + 5% polyethylene oxide 1M with 30% metoprolol tartrate), initially ( ) and after storage for 1 month at 25°C/60%RH ( ) and 40°C/75%RH ( ), 3 months at 25°C/60%RH ( ) and 40°C/75%RH ( ) and 6 months at 25°C/60%RH ( ) and 40°C/75%RH ( ). Mean (n = 3) dissolution profiles (± SD) of co-extrudates. Dissolution at 37 °C and 100 rpm.

MDSC-profiles for the core extrudates after 1 and 3 months at different storage conditions indicated the stability of the crystalline state of the metoprolol tartrate fraction incorporated in the core. To evaluate the physical stability of the coat the X-ray patterns of the 5.6 % hydrochlorothiazide formulation stored during 3 months at different storage conditions were compared with the X-ray pattern of the physical mixture and of the pure drug (Fig. 9). The diffraction patterns of the 3 months stability samples were similar with the formulation immediately after processing and indicated that hydrochlorothiazide stayed dissolved in the coat after 3 months at both conditions.

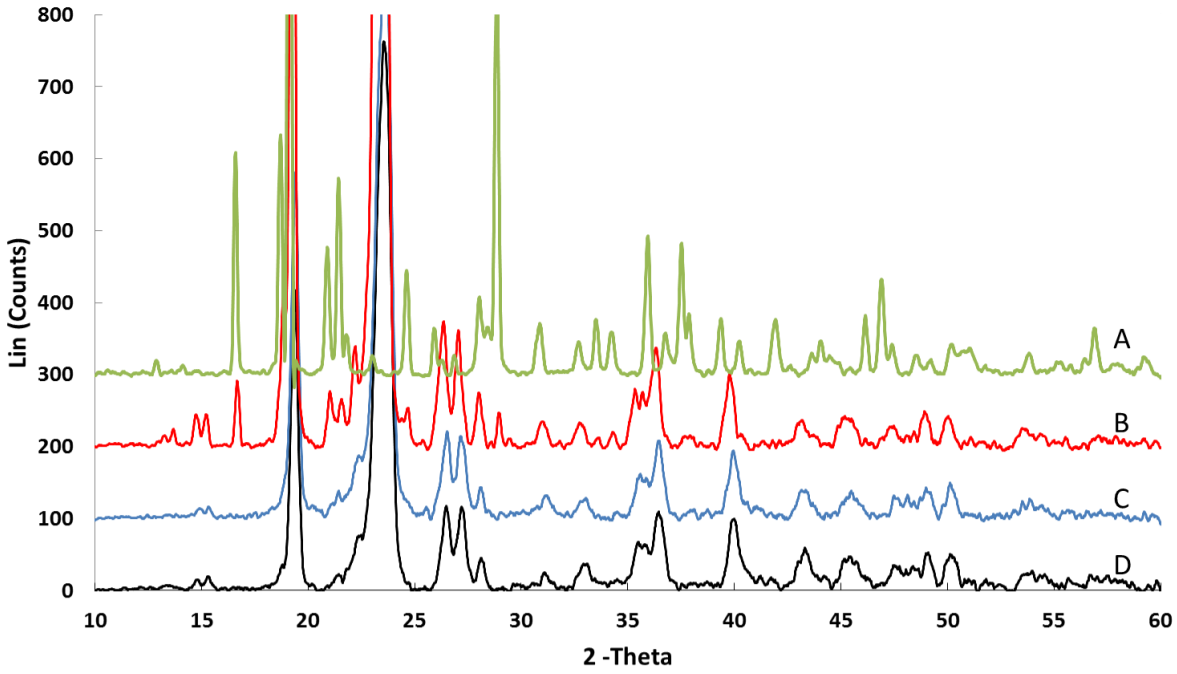

Figure 9. X-ray diffraction patterns of hydrochlorothiazide (A), physical mixture coat (B), coat co-extrudate after 3 months storage at 25°C/60%RH (C) and coat co-extrudate after 3 months storage at 40°C/75%RH (D) for formulation A.

In vivo evaluation

To study the hydrochlorothiazide and metoprolol tartrate bioavailability, co-extrudates (formulation A, B, C and D) were administered to 6 dogs. The bioavailability was compared with a commercially available reference formulation, Zok-Zid®, a tablet containing hydrochlorothiazide and coated metoprolol tartrate pellets. The mean normalized plasma concentration-time profiles (n = 6) for hydrochlorothiazide and metoprolol tartrate after oral administration of formulation A, B, C (25 mg hydrochlorothiazide and 200 mg metoprolol tartrate), formulation D (25 mg hydrochlorothiazide and 100 mg metoprolol tartrate) and the reference (2 tablets) are illustrated in Fig. 10 and Fig. 11, respectively, while the mean pharmacokinetic parameters (AUC, C_{max}, t_{max} and $HVD_{t50\%Cmax}$) are reported in Table 2 and Table 3, respectively. The drug plasma concentration profiles confirmed the *in vitro* dissolution results. The immediate *in vitro* hydrochlorothiazide release from the coat was confirmed *in vivo* with a T_{max} value of about 2 hours for all co-extrudates. The rather fast *in vitro* metoprolol tartrate release from formulation A was reflected in the *in vivo* study since this formulation showed a burst release with a mean C_{max} of 26.6 ng/ml obtained after 2.8 h, compared with a C_{max} of 12.8 ng/ml and 12.3 ng/ml obtained after 3.1 h and 3.5 h after administration of formulation B and C, respectively. The high metoprolol tartrate plasma concentrations reached after administration of the co-extruded matrix formulations in comparison to the reference formulation, were indicating that the administered MPT dose could be reduced. Therefore, Formulation D was designed with the same core composition as formulation B in combination with a coat having a higher HCT load to be able to half the MPT dose while maintaining the HCT dose for administration to dogs. The pharmacokinetic parameters (AUC and C_{max}) of this formulation were not different at the 0.05 level of

significance from the reference formulation. Moreover, similar to formulation B and C, formulation D was characterized by a $HVD_{t50\%Cmax}$ of more than 7 h, illustrating better controlled release characteristics than the reference formulation with a $HVD_{t50\%Cmax}$ of 4.8 h. The difference in pharmacokinetic parameters for metoprolol tartrate between the co-extruded matrix formulations and the coated pellet reference formulation, as already noticed in the *in vitro* results, can be observed clearly *in vivo*, where a lag phase of 2 hours is seen for the reference formulation. This might be attributed to the hydrophobic ethylcellulose coating of the pellets in comparison to the mini-matrices, causing metoprolol tartrate only to be released further down the gastrointestinal tract (GIT) and thus missing the absorption sites in the dogs small intestine. It has been documented that dogs have an unsacculated colon and therefore handle the transit of fluid and small particles in a fast way [20]. In contrast to the small pellets in the reference formulation it can be assumed that the larger mini-matrices are retained at the ileocecal junction, increasing the residence time in the ileum which offers a better absorption of the metoprolol tartrate, since it has been demonstrated previously that metoprolol is absorbed to the same degree from the duodenum and colon [21]. It has been demonstrated that for metoprolol tartrate the co-extruded matrix formulations offer a higher bioavailability in dogs (AUC 3 to 7-fold higher and C_{max} 2 to 7-fold higher) than the reference pellet formulation. From the dog plasma concentrations it can be concluded that the metoprolol tartrate dose administered with formulation D could even be halved.

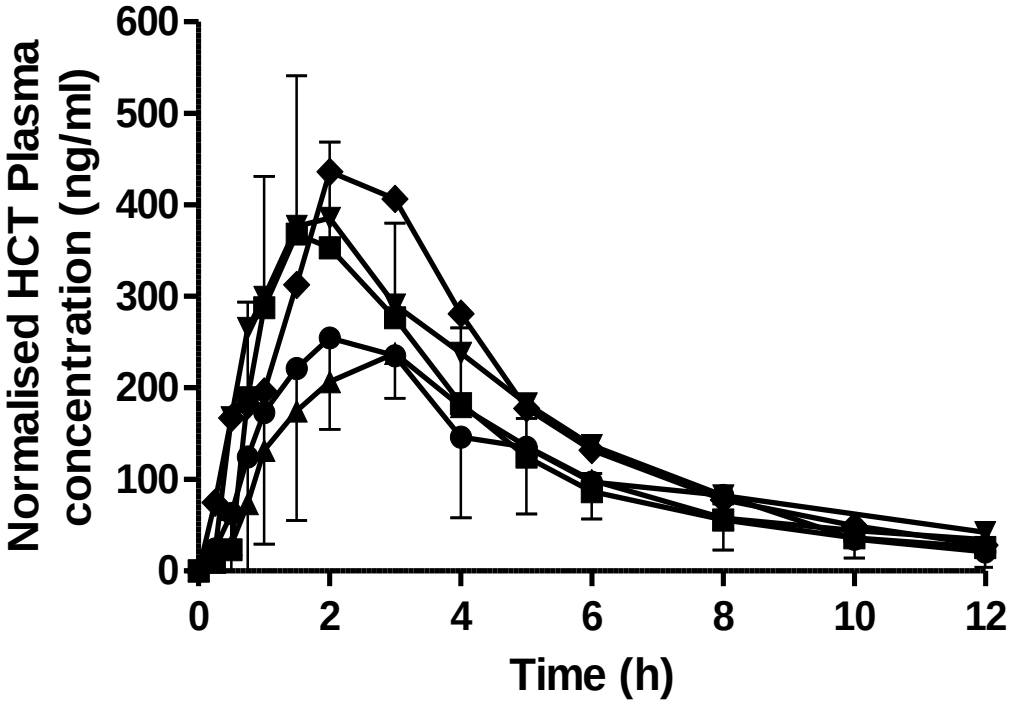

Figure 10. Mean (n = 6) hydrochlorothiazide (HCT) plasma concentration-time profiles after oral administration of the co-extruded formulations A ( ), B ( ), C ( ), D ( )
(25 mg hydrochlorothiazide) and Zok-Zid® tablets ( ) (2 tablets) to dogs.
The SD for formulation A and B are shown, for the other formulations SD is of the same magnitude.

HCT	AUC0-12h (µg.h/ml)	Cmax (µg/ml)	Tmax (h)
Co-extrudate A	1,3 ± 0,4^{a,b}	0,3 ± 0,1^{a}	2,3 ± 0,9
Co-extrudate B	1,6 ± 0,5^{a,b}	0,4 ± 0,1^{a,b}	1,9 ± 0,6
Co-extrudate C	1,3 ± 0,2^{a}	0,3 ± 0,0^{a}	2,5 ± 1,0
Co-extrudate D	2,0 ± 0,3^{b}	0,5 ± 0,2^{a,b}	1,9 ± 1,0
Reference	2,0 ± 0,2^{b}	0,6 ± 0,1^{b}	2,5 ± 1,1

a, b: means in the same column with the same superscript are not different at the 0.05 level of significance.

Table 2. Mean (n = 6) pharmacokinetic parameters (± SD) after oral administration of the co-extruded formulations A, B, C, D (25 mg hydrochlorothiazide) and Zok-Zid® tablets (2 tablets) to dogs.

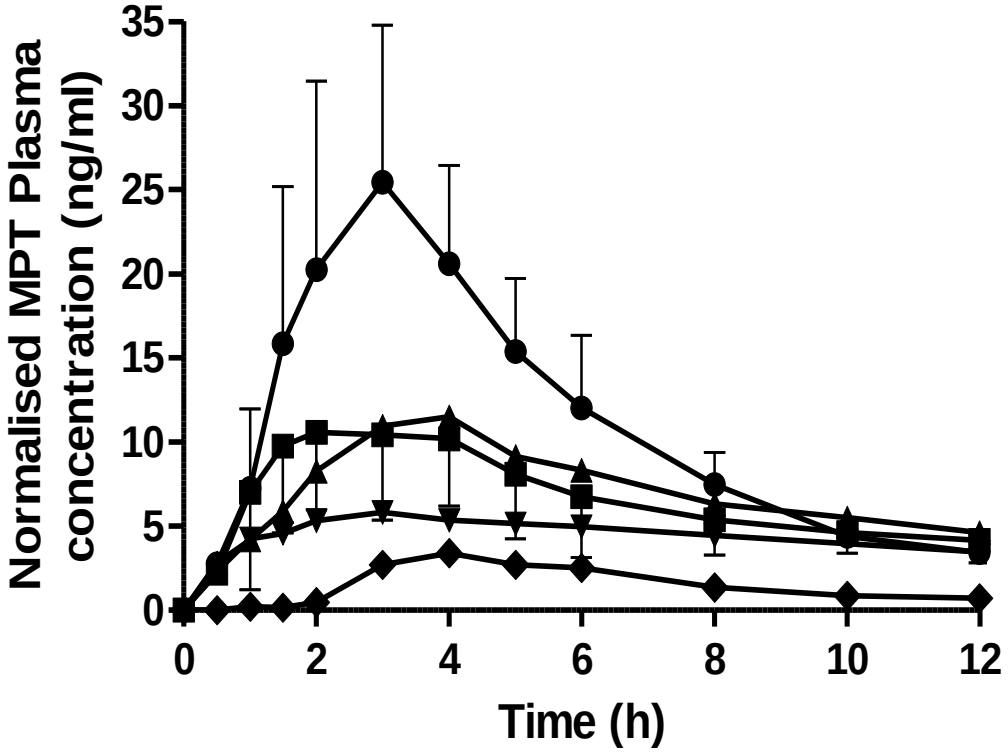

Figure 11. Mean (n = 6) metoprolol tartrate (MPT) plasma concentration-time profiles after oral administration of the co-extruded formulations A ( ), B ( ), C ( ) (200 mg metoprolol tartrate) and D ( ) (100 mg metoprolol tartrate) and Zok-Zid® tablets ( ) (2 tablets) to dogs. The SD for formulation A and B are shown, for the other formulations SD is of the same magnitude.

MPT	AUC0-12h (ng.h/ml)	Cmax (ng/ml)	Tmax (h)	HVDt50%Cmax (h)
Co-extrudate A	134,7 ± 43,2^{c}	26,6 ± 9,2^{d}	2,8 ± 0,8	4,4
Co-extrudate B	88,9 ± 35,0^{a,b,c}	12,8 ± 5,6^{a,c}	3,1 ± 0,9	8
Co-extrudate C	85,0 ± 18,2^{b,c}	12,3 ± 3,6^{b,c,d}	3,5 ± 0,5	7,8
Co-extrudate D	53,6 ± 11,0^{a,b}	7,4 ± 2,0^{a,b}	4,5 ± 3,9	>11,4
Reference	18,3 ± 14,7^{a}	3,8 ± 3,3^{a}	4,7 ± 2,0	4,8

a, b, c, d: means in the same column with the same superscript are not different at the 0.05 level of significance.

Table 3. Mean (n = 6) pharmacokinetic parameters (± SD) after oral administration of the co-extruded formulations A, B, C (200 mg metoprolol tartrate) and D (100 mg metoprolol tartrate) and Zok-Zid® tablets (2 tablets) to dogs.

Mathematical modeling

A new mathematical model was developed to predict the drug plasma profiles as a function of the observed *in vitro* drug release kinetics, taking into account: (a) metoprolol tartrate transport within the controlled release dosage form and (b) drug fate *in vivo*. The theoretical predictions were compared with independent experimental results. The rational of the mathematical theory is as follows: Drug release from the ethylcellulose matrix was described using a mechanistically realistic theory, assuming that drug diffusion in the cylinders is the dominant mass transport step (Fig. 12). Considering perfect sink conditions, an initial homogeneous drug and polymer distribution and unaltered device dimensions during drug release, Fick's second law of diffusion can be solved using the method of Laplace transformation, leading to [22]:

$$\frac{M_t}{M_\infty} = 1 - \frac{32}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{q_n^2}exp\left(-\frac{q_n^2}{R^2}Dt\right)\cdot\sum_{p=0}^{\infty}\frac{1}{(2p+1)^2}\cdot exp\left(-\frac{(2p+1)^2\pi^2}{H^2}Dt\right)$$

where M_t and M_∞ denote the cumulative amounts of drug released at time t and at infinite time, respectively; D is the diffusion coefficient of the drug within the system; the q_n are the roots of the Bessel function of the first kind of zero-order [$J_0(q_n)=0$]; n and p denote dummy variables, and R and H represent the radius and height of the cylinder, respectively.

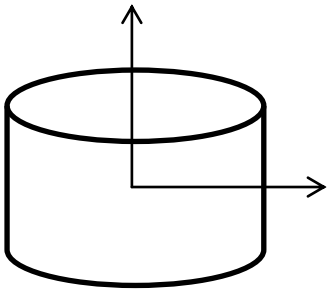

Figure 12. Radial and axial diffusional drug transport in the cylindrical dosage form, considered in the mathematical model.

Fitting this equation to the experimentally measured metoprolol tartrate release kinetics from different types of formulations resulted in good agreement between theory (curves in Fig. 13) and experiment (symbols in Fig. 13).

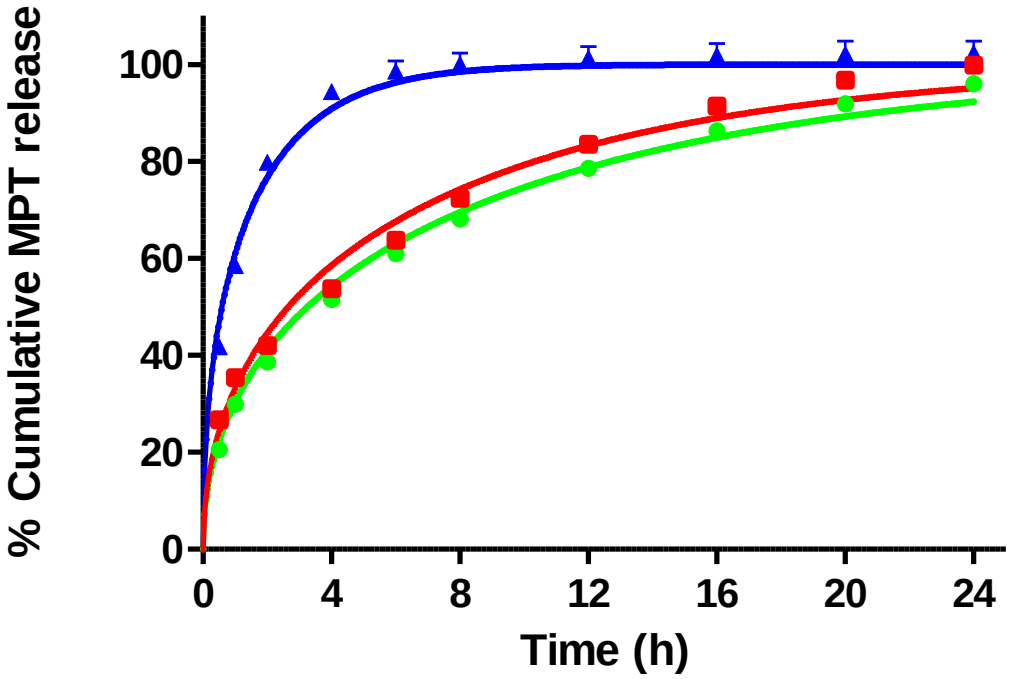

Figure 13. Theoretical predictions (curves) and independent experimental results (symbols) of *in vitro* metoprolol tartrate release for formulation A (blue, ▲), formulation C (green, ●) and formulation D (red, ■).

The model assumes that *in vitro* drug release is identical to drug release within the gastrointestinal tract, the dosage form being robust and controlling drug release in the same manner in the different surroundings.

Once released the drug is available for absorption into the blood stream. An open one-compartment model is assumed, with first order absorption and first order elimination kinetics (Fig. 14).

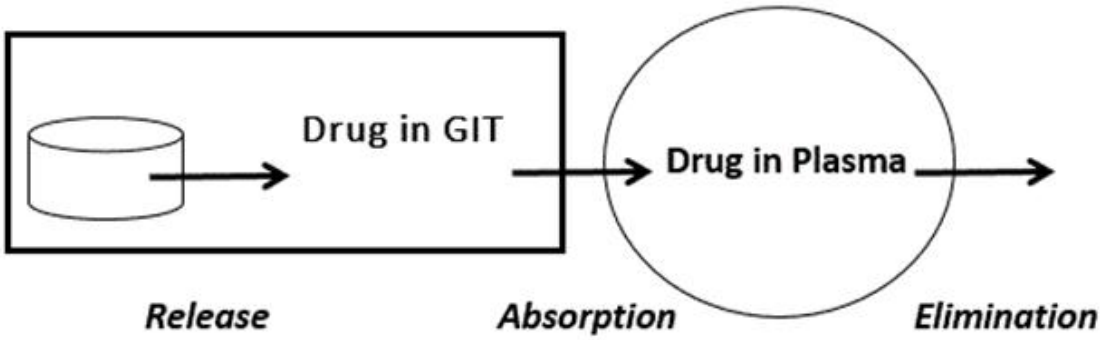

Figure 14. Schematic representation of the processes taken into account by the applied mathematical model, considering drug release in the gastrointestinal tract (GIT), drug absorption into the blood stream and drug elimination.

The following parameters of metoprolol were taken from literature:

(i) absorption rate constant reported in humans for a "fast releasing formulation" (0.85 1/h) [23],

(ii) volume of distribution/bioavailability factor reported in humans for a "fast releasing formulation" (638 L) [23],

(iii) elimination rate constant reported in beagle dogs (0.4 1/h) [24].

The mathematical model was solved numerically, using the programming language C++. Fig. 15 shows a comparison of the resulting metoprolol tartrate plasma profiles: the curves

represent the theoretically predicted pharmacokinetics, the symbols the independent experimental results (Fig. 15).

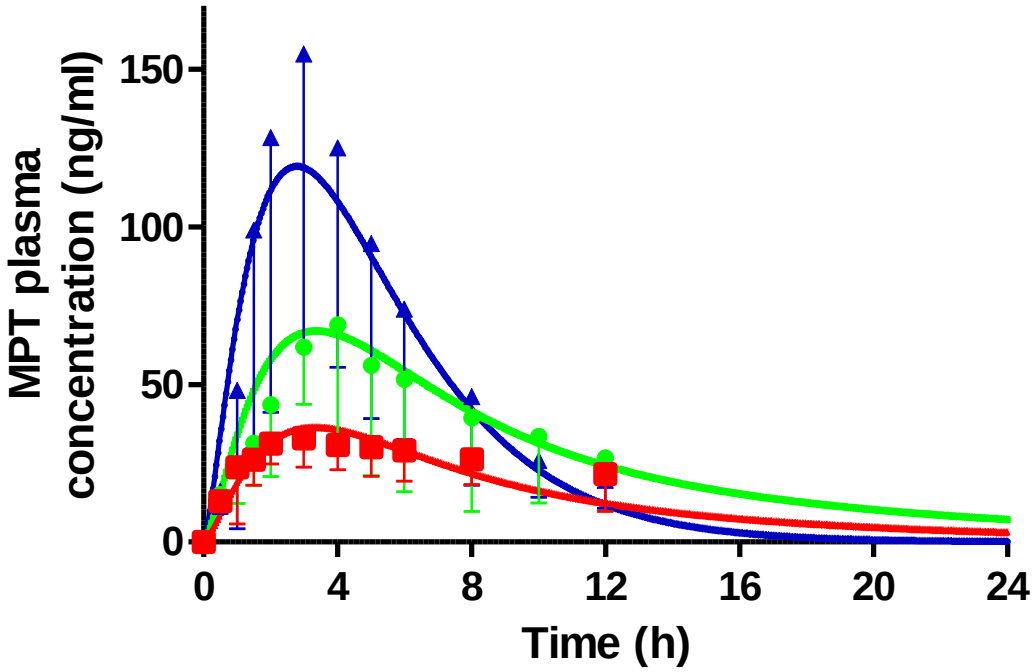

Figure 15. Theoretical predictions (curves) and independent experimental results (symbols) of metoprolol tartrate plasma profiles for formulation A (blue, ▲), formulation C (green, ●) and formulation D (red, ■).

The good agreement between theoretical predictions and independent experimental results indicated that the developed mathematical model is applicable and that the resulting *in vivo* drug plasma levels can be predicted based on *in vitro* release kinetics. This can reduce the number of *in vivo* studies required for product optimization.

CONCLUSION

In this chapter we have demonstrated that fixed-dose combination mini-matrices with a matrix core offering a range of controlled release profiles and an immediate release coat were successfully developed by co-extrusion. The mini-matrices showed good adhesion and no migration of active drug substances between core and coat. Metoprolol tartrate release from the ethylcellulose matrix core could be sustained in function of the drug load and the content of the hydrophilic additive. High metoprolol tartrate plasma concentrations were obtained after oral administration to dogs, which indicated that the MPT dose could be lowered to achieve the same bioavailability compared to a commercial reference formulation. However this advantage in dose reduction should be confirmed in humans. A stability study indicated that the co-extrudates were stable for at least 6 months at different storage conditions. From this study it can be concluded that co-extrusion proved to be a valuable technique for the production of oral FDC drug products with multiple release profiles. Moreover the featured tests provided a thorough physical characterization of the co-extrudates manufactured. Interestingly, an appropriate mathematical model (taking into account drug transport within the controlled release dosage form and the *in vivo* fate of metoprolol tartrate) was able to predict the resulting drug plasma levels, based on the *in vitro* release kinetics and parameters reported in the literature. The theoretical predictions agreed well with independent experimental results. Thus, the number of *in vivo* studies required for product optimisation can be reduced.

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CHAPTER 2

CALENDERING AS A DIRECT SHAPING TOOL FOR THE CONTINUOUS PRODUCTION OF FIXED-DOSE COMBINATION PRODUCTS VIA CO-EXTRUSION

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ABSTRACT

In this chapter calendering is used as a downstream technique to shape monolithic co-extruded fixed-dose combination products in a continuous way. Co-extrudates with a metoprolol tartrate-loaded sustained-release core and a hydrochlorothiazide-loaded immediate-release coat were produced and immediately shaped into a monolithic drug delivery system via calendering, using chilled rolls with tablet-shaped cavities. *In vitro* metoprolol tartrate release from the ethylcellulose core of the calendered tablets was prolonged in comparison to the sustained release of a multiparticulate dosage form, prepared manually by cutting co-extrudates into mini-matrices. Analysis of the dosage forms using X-ray micro-computed tomography only detected small differences between the pore structure of the core of the calendered tablet and the mini-matrices. Diffusion path length was shown to be the main mechanism behind the release kinetics. Terahertz pulsed imaging visualized that adhesion between the core and coat of the calendered tablet was not complete and a gradient in coat thickness (varying from 200 to 600 µm) was observed. Modulated differential scanning calorimetry and X-ray diffraction indicated that the physicochemical properties of both drugs were not affected by the calendering procedure.

CHAPTER 2

CALENDERING AS A DIRECT SHAPING TOOL FOR THE CONTINUOUS PRODUCTION OF FIXED-DOSE COMBINATION PRODUCTS VIA CO-EXTRUSION

INTRODUCTION

In co-extrusion two or more formulations are simultaneously processed via hot-melt extrusion (HME) through the same die. In addition to the advantages of HME, such as the continuity of the production process, not requiring the use of solvents or water and improving drug bioavailability, this technique offers the opportunity to produce fixed-dose combination (FDC) products with enhanced release characteristics, by making it possible to design multilayered dosage forms that are extruded in the same process step, in order to modulate the drug release from each layer. Although co-extrusion is used to manufacture implants [1] and vaginal rings [2], there are currently no co-extruded dosage forms for oral application on the market. In the literature only a limited number of studies describe co-extrusion of dosage forms for oral drug delivery [3-6]. Recently co-extrusion has been used for the development of multiparticulate fixed-dose combination drug products for oral pharmaceutical application, consisting of a controlled release core matrix and an immediate

release coat [7]. For pharmaceutical applications of co-extrusion, one of the major challenges is the shaping of the final product in a continuous way, as a suitable downstream shaping technique is needed to ensure an efficient manufacturing line. Previously injection molding has been used to shape extrudates into solid oral dosage forms in a semi-continuous way [8, 9] and even to prepare co-injection moulded matrices [4]. Calendering is a technique that allows in-line shaping of the extruded material in a fully continuous single-step process. Using this technique the freshly-extruded thermoplastic strand is guided through a pair of temperature-controlled rolls containing tablet- or pill-shaped cavities, yielding strips that contain single tablet-shaped cores of the desired shape. Although this technique is already widely established in the plastic and confectionary industry to produce monolithic shapes, only the Meltrex® technology [10] and the continuous extrusion process for the production of sustained release tablets developed by Knoll AG [11] report calendering as a possible shaping tool for pharmaceutical applications.

In this study the use of calendering to continuously shape a multilayered co-extrudate into a monolithic FDC dosage form was evaluated. In the treatment of cardiovascular disease the FDC of the beta-blocker metoprolol tartrate (MPT) with the diuretic hydrochlorothiazide (HCT) is well established [12]. Therefore co-extrudates consisting of a plasticized ethylcellulose (EC) core, containing MPT and polyethylene oxide (PEO), and a coat of polyethylene oxide (PEO)/polyethylene glycol (PEG) containing HCT were produced. Afterwards the cylindrical co-extrudate with concentric coat layer was immediately shaped via calendering, using chilled rolls with tablet-shaped cavities. In this way monolithic dosage forms with a sustained-release core, loaded with MPT as model drug, and an immediate-release coat, loaded with HCT as model drug, were produced and evaluated for *in vitro* drug release, coat thickness and uniformity and pore structure. The impact of the calendering

step on the physical state of the drugs in the formulations was characterized using modulated differential scanning calorimetry (MDSC) and X-ray diffraction (XRD).

MATERIALS AND METHODS

Materials

Metoprolol tartrate (MPT) (Esteve Quimica, Barcelona, Spain) and hydrochlorothiazide (HCT) (Utag, Amsterdam, the Netherlands) were used as sustained and immediate release model drugs, respectively. As excipients ethylcellulose (EC) (Ethocel® std 10, Colorcon, Dartford Kent, United Kingdom), dibutyl sebacate (DBS) (Sigma-Aldrich, Bornem, Belgium), polyethylene oxide (PEO) 1M (MW: 1000000 g/mol, Sentry™ Polyox® WSR N12K, Colorcon, Dartford Kent, United Kingdom), PEO 100K (MW: 100000 g/mol, Sentry™ Polyox® WSR N10, Colorcon, Dartford Kent, United Kingdom) and polyethylene glycol (PEG) 4K (MW: 4000 g/mol, Fagron, Waregem, Belgium) were used. All other chemicals were of analytical grade.

Hot-melt co-extrusion

Co-extrusion was carried out using two co-rotating Prism Eurolab 16 mm twin screw extruders (ThermoFisher Scientific, Karlsruhe, Germany), connected to a co-extrusion die (Guill, West Warwick, USA). In the calendering set-up the co-extrusion die was adapted to fit the diameter of the co-extrudate with the dimensions of the calender cavities, shaping a cylindrical co-extrudate consisting of a core with a diameter of 4 mm and a concentric coat with a thickness of 2 mm. To produce the multiparticulates, a cylindrical co-extrudate with an inner diameter of 3 mm and an outer diameter of 4 mm was manufactured. The heating zones of both extruders were heated to 80/90/100/100/100/100 °C from feed opening to die-end. The co-extrusion die was heated to 100 °C. Both premixes were fed separately into an extruder by a Brabender Flexwall® loss-in-weight powder feeder (Brabender, Duisburg,

Germany) at a feed rate of 200 g/h for the coat and 300 g/h for the core material. A screw speed of 40 rpm and 150 rpm was used for the extruder producing the outer layer and the inner layer, respectively.

Downstream processing

Calendering was performed with a Collin 60 mm calender (Dr. Collin, Ebersberg, Germany), coupled to a compressed air supply and a Coolenergy chiller (Plastima, Breda, The Netherlands), which cooled the calender rolls to a temperature within the range of 4-8 °C. The speed of the calender rolls was set at 1.5 rpm. Immediately after leaving the co-extrusion die the co-extruded strand was guided between a pair of chilled pressurized rolls that contained tablet-shaped cavities, yielding tablets with a diameter of 8 mm and a thickness of 5 mm (cfr. Fig. 5 page 22).

To test the effect of cooling on the MPT release a core extrudate was prepared using the same process parameters as for the core in the co-extrudate. Part of this material was cooled at room temperature, while the remaining part was quench-cooled by dipping the core extrudate in liquid nitrogen immediately after extrusion.

Multiparticulates were obtained by manually cutting a cylindrical co-extrudate with an inner diameter of 3 mm and an outer diameter of 4 mm into mini-matrices of 2 mm length after cooling the co-extruded rod to room temperature.

In vitro drug release

In vitro dissolution was performed using United States Pharmacopeia (USP) dissolution apparatus 1 (baskets) on an Evolution 6300 dissolution system (Distek, New Brunswick, New

Jersey, USA), coupled with an Evolution 4300 automatic dissolution sampler (Distek, New Brunswick, New Jersey, USA). The temperature of the dissolution medium (900 ml) was kept at 37 ± 0.5 °C and the rotational speed of the baskets was set to 100 rpm. For the first hour a 0.1 N solution of hydrochloric acid (pH 1) was used as the dissolution medium. Afterwards the baskets containing the mini-matrices or tablets were transferred to vessels filled with phosphate buffer pH 6.8 (USP) as the dissolution medium. Samples (filtered using Distek 45 µm filters) of 5 ml were withdrawn at 5, 10, 15, 20, 30, 45 and 60 minutes for the determination of HCT concentration in the first dissolution medium and at 1, 2, 4, 6, 8, 12, 16, 20 and 24 hours for the determination of MPT concentration in the second dissolution medium. The core layer was analyzed separately to cover for the MPT release during the first hour. Samples were analyzed spectrophotometrically at 316.6 and 222.0 nm, using a UV-spectrophotometer, type UV-1800 (Shimadzu, Deurne, Belgium) and applying an appropriate calibration curve for quantification of HCT and MPT, respectively. Each experiment was performed in triplicate.

Modulated differential scanning calorimetry

The solubility of HCT in the coat of the tablet was studied by cyclic heating of an oversaturated sample, containing 70 % HCT, followed by annealing at a different temperature for each cycle in order to reach the maximum solubility at each temperature. After the annealing step the sample was quenched and heated again to determine the glass transition temperature (*Tg*). These cycles were performed for different annealing temperatures in between the melting point of polymer matrix and drug, and the shift in *Tg* was monitored using a differential scanning calorimeter Q200, equipped with a refrigerated cooling system (RCS) (TA Instruments, Leatherhead, UK). Nitrogen was used as purge gas

through the DSC cell (50 ml/min) and the RCS unit (300 ml/min). Samples (± 3 mg) were run in an open aluminum pan with an underlying heating rate of 5 °C/min. The modulation period and amplitude were set at 50 s and 0.663 °C, respectively (heat-only method). Temperature and enthalpy calibration was performed with an indium standard at the same scan rate and with the same kind of pans used in the experiment. MDSC data were analyzed using the TA instruments Universal Analysis 2000 V4.7A software.

X-ray diffraction

Crystallinity was analyzed using X-ray diffraction (XRD) on pure compounds, physical mixtures and corresponding extrudates. X-ray diffraction was performed on a D5000 diffractor with Cu Kα radiation (λ = 1.54 Å) (Siemens, Karlsruhe, Germany) and a voltage of 40 mV in the angular range (2θ) varying from 10 to 60 ° using a step scan mode with a step size of 0.02 ° and a measuring time of 1 s/step.

Terahertz pulsed imaging

The calendered tablets from different formulations were analyzed using a terahertz pulsed imaging (TPI) imaga2000 coating scan system (Teraview, Cambridge, UK). The operation of this system was previously described by Zeitler et al. [13]. Images were acquired in a point-to-point mode with a step size of 200 µm. Images were analyzed using TPI View (version 3.0.3, Teraview, Cambridge, UK). A six-axis robot arm was used to produce a surface map of the calendered tablet. The refractive index of the coating material was estimated to be 1.5, based on the surface reflectivity of the calendered tablets as well as by calibration using X-

ray micro-computed tomography. Given the very smooth texture of the surface this was deemed to represent an appropriate measurement for the refractive index as no surface scattering would contribute to the losses. Using this value histograms and maps of coating uniformity were plotted using Matlab (R2013a, The Mathworks, Natick MA, USA).

X-ray micro-computed tomography

The porosity of the mini-matrices and tablets was evaluated by means of X-ray micro-computed tomography (micro-CT). Co-extruded mini-matrices and calendered tablets were scanned using a Skyscan 1172 high resolution X-ray micro-CT system (Bruker microCT, Kontich, Belgium), operated at 59 kV source voltage, with an image pixel size of 1.37 µm and 4.53 µm, for the mini-matrix and the tablet, respectively. The scanning system is equipped with an aluminum 0.5 mm filter and an 11 Mp charge coupled device (CCD) detector. For the scan with the image pixel size of 4.53 µm the samples were rotated over 0.4 ° steps, exposure time was 1000 ms and total scan duration was 42 min. For the high resolution offset-scan the samples were rotated over 0.2 ° steps, exposure time was 2350 ms, frame averaging was 5 and total scan duration was 9 h 17 min. The images were reconstructed with NRecon (Version 1.6.3.2, Bruker microCT, Kontich, Belgium) on a GPU-ReconServer. A Gaussian smoothing kernel of 2 pixels was applied, resulting in an 8-bit bitmap (BMP) image with a linear X-ray attenuation coefficient, displayed as a grey scale value calibrated between 0 and 255. To compare both dosage forms at the same pixel size, the images of the mini-matrix system were resized fourfold prior to analysis. Data analysis and visualization was done with CTAn software (version 1.13.5.1, Bruker microCT, Kontich, Belgium) and CTVol (version 2.2, Bruker microCT, Kontich, Belgium) for surface rendering. For image

analysis the core was defined as the region of interest (ROI). To this end, applying a Gaussian blur by 2 pixels allowed separating the two peaks in the grey scale histogram with a threshold of 34. Pixels with lower intensities were assigned to the core and pixels with a higher intensity were assigned to the coat layer. This ROI is applied to the original greyscale images, in this way removing the coat. Greyscale images were binarised using an Otsu-algorithm, one of the most popular techniques of automatic thresholding [14]. 3D objects smaller than 20 voxels were considered to be noise and were filtered out of the image used for porosity analysis. A distinction is made between internal pores, which are located in the core of the co-extrudate, and pores at the interface between core and coat. The percentage of internal pores is quantified as the ratio between the internal pore volume in the core and the object volume (i.e. total volume of solid core material, excluding pores). The percentage of pores at the interface between both layers is defined as the ratio between the pore volume at the interface and the total core volume (i.e. region of interest volume, including pores). A size distribution of the pores is illustrating the percentage of pores in a certain range of structure thickness. Local structure thickness for a point in solid material is defined by Hildebrand and Ruegsegger as the diameter of the largest sphere that encloses the point and is entirely enclosed within the solid surfaces [15].

RESULTS AND DISCUSSION

In order to continuously shape a multilayered co-extrudate into a final monolithic FDC tablet-shaped dosage form the co-extrusion line was extended downstream with a calender and chiller. Although calendering has been used previously to shape an extrudate into a final dosage form [10, 11], to our knowledge this study is the first to evaluate calendering as a downstream tool in a co-extrusion process, thus producing a dosage form with an outer layer that is surrounding the inner core. In order to evaluate the effect of calendering on the release profiles of two model drugs, formulations with a MPT-loaded sustained release core and a HCT-loaded immediate release coat were used (Table 1): the two core formulations A and B varied in their EC/PEO ratio while a lower MPT content was used in formulation C. This allowed to evaluate the effect of calendering as a function of the concentration of PEO (added as hydrophilic additive) and the MPT load [16]. The composition of the coat formulation was constant, except for the HCT load of formulation C that was adjusted in order to respect the same MPT/HCT ratio in each of the co-extruded formulations.

		Matrix			Drug load
Formulation A	Core	53% EC	27% DBS	20% PEO 1M	30% MPT
	Coat	85% PEO100K		15% PEG 4K	5.6% HCT
Formulation B	Core	62% EC	33% DBS	5% PEO 1M	30% MPT
	Coat	85% PEO100K		15% PEG 4K	5.6% HCT
Formulation C	Core	53% EC	27% DBS	20% PEO 1M	15% MPT
	Coat	85% PEO100K		15% PEG 4K	2.8% HCT

Table 1. Composition of calendered formulations A, B and C.

The MPT-loaded plasticized EC matrix (with the addition of PEO 1M as a hydrophilic additive) was co-extruded with its HCT-loaded PEO 100K/PEG 4K coat at a temperature of 100 °C. The inserts of the co-extrusion die were adapted to match the dimensions of the co-

extrudate with the dimensions of the calender cavities. The co-extruded string was guided between the chilled calender rolls to shape a string of tablets. Calendered tablets were regular in shape and had a uniform aspect as long as the calender rolls were chilled at 4 ± 1 °C. When the chiller did not succeed in adequately cooling the calender rolls (i.e. using a chill water temperature > 6°C), the calendered tablets deformed when the calendered string detached from the calender rolls. As aspect defects were found to be highly dependent on calender speed and temperature, a low calender speed was used to allow adequate cooling and perfect shaping of the calendered tablets [17]. The cooling rate of the material between the calender rolls seemed essential to obtain a dosage form with good shape and uniform dimensions (Fig. 1).

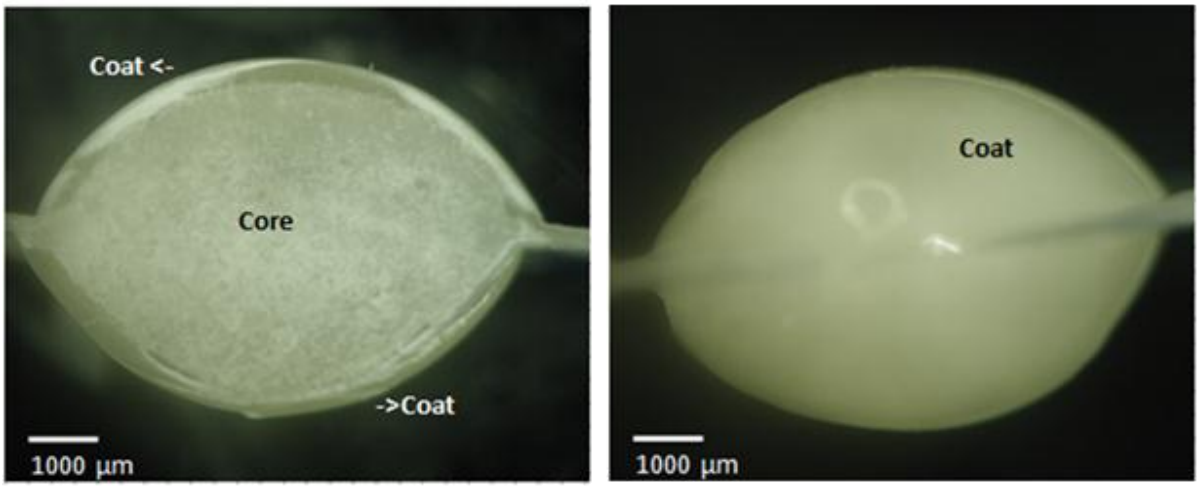

Figure 1. Cross-section (left) and side-view (right) of a calendered tablet, with a sustained release core and an immediate release coat.

Although minimized by adjusting the die dimensions to the dimensions of the calender cavities, waste material was created at the sides of the tablets when forcing the co-extruded strand in between the calender rolls. The amount of waste was 7.5 % w/w of the total weight of the co-extruded calendered material. As this waste at the edges of the tablets

must be removed to obtain the final dosage forms, this is especially a disadvantage when working with highly valuable active ingredients. Based on the drug content of the waste material, it was assessed that it is mainly composed of coat material (93 % of the total waste fraction), while only a minor part of core material is lost during calendering. This should be taken into account as this yields tablets with a higher MPT/HCT ratio than theoretically anticipated based on coat and core composition. The higher MPT/HCT ratio was confirmed by quantification of the MPT and HCT concentration in the calendered tablets: a ratio of 8.58 vs. 8.00 based on the composition of coat and core layer. Since TPI was previously used for the non-destructive analysis of coated tablets [18] this technique was used to visualize the coat layer and to study the adhesion between core and coat. Especially interesting for the analysis of calendered tablets via TPI is the fact that not only the thickness of the coat layer, but also the uniformity and integrity of the coat can be analyzed, since penetration depths into typical pharmaceutical formulations between 1 and 3 mm can currently be achieved [13]. The thickness of the coat layer varied between 200 and 600 µm (Fig. 2).

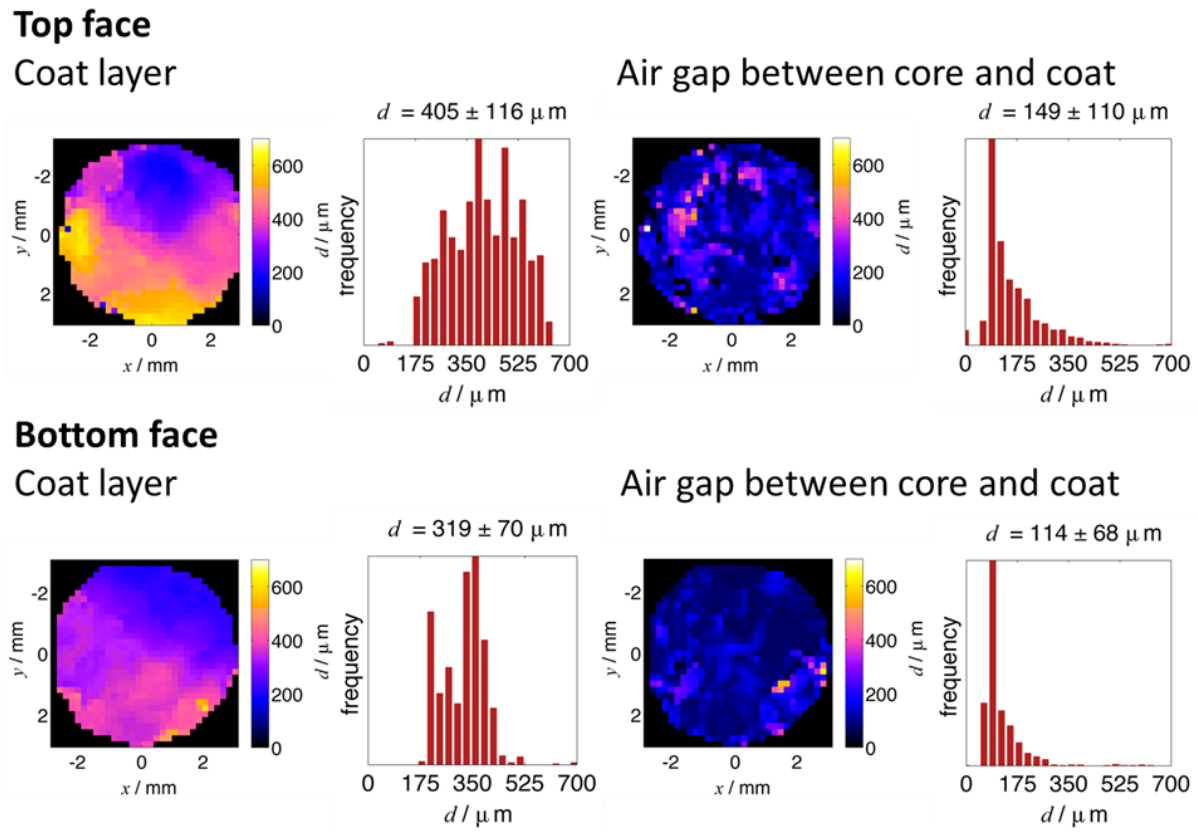

Figure 2. False-colour images, showing spatial distribution, and histograms of coating thickness for coat layer and for the air gap between core and coat layer, of the calendered tablet formulation B, analyzed both at top and bottom of the tablet.

In the false-colour images of the tablets of formulation B, a thickness gradient in the coat layer is observed (Fig. 2), which is more pronounced at the top face compared to the bottom face of the calendered tablets. This gradient can be explained by the sequenced contact of different regions of the tablet with the calender rolls. The cross section images based on the micro-CT data from a string of tablets confirmed this by revealing a recurrent pattern of the thinner area in the coating layer (Fig. 3).

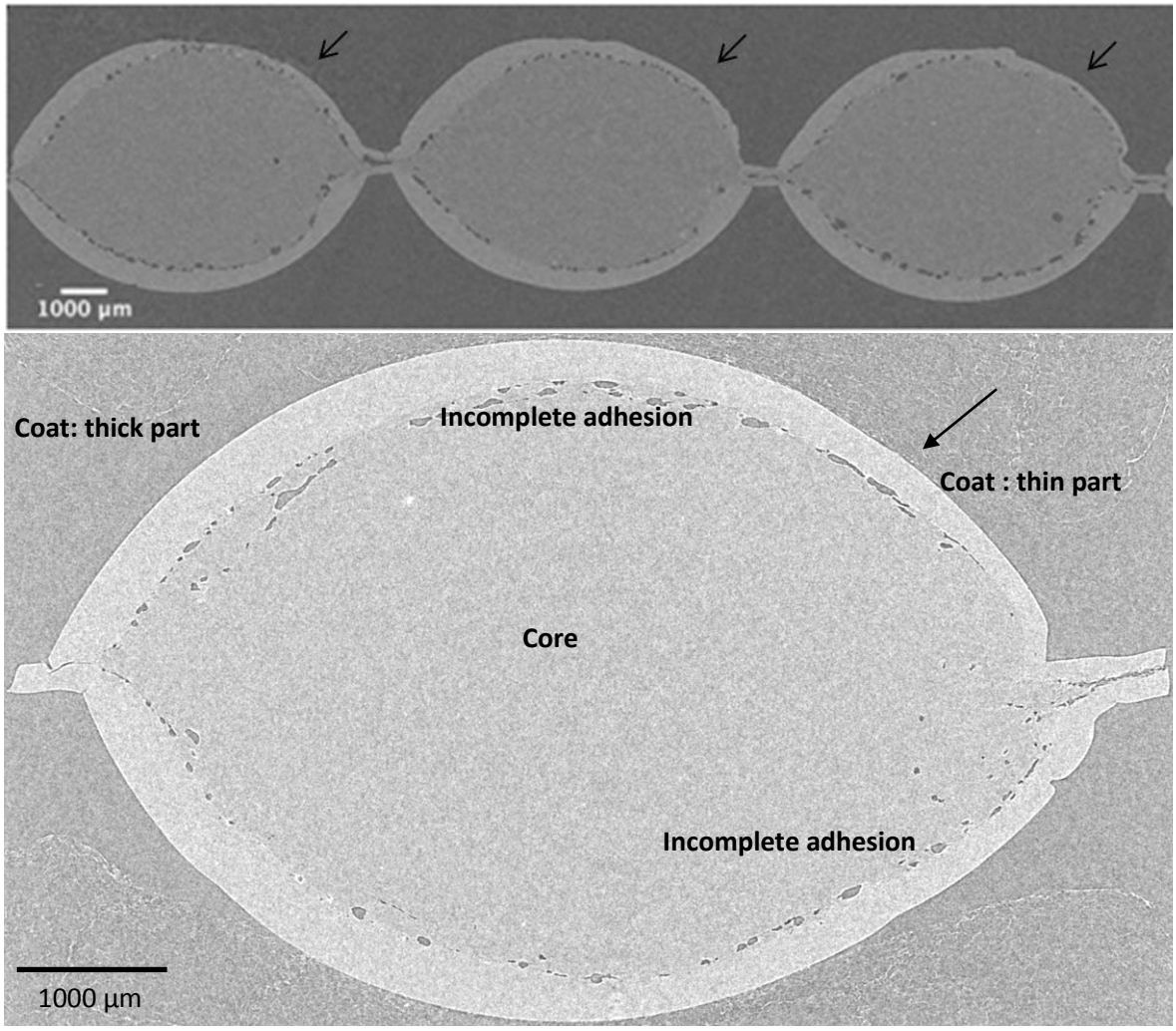

Figure 3. Micro-CT image of a string of calendered tablets, where the recurrent thinner part of the coat layer is indicated with an arrow and detail of the calendered tablet for formulation B.

The analysis of the calendered tablet via TPI also clearly identified that the adhesion between core and coat was not complete as an air gap at the interface between both extruded layers was detected (Fig. 2). The incomplete adhesion was also confirmed by micro-CT (Fig. 3) and quantified as the percentage of pores at the intersection of core and coat. These pores at the intersection of core and coat for calendered tablets (2.37 %) were also found for the multiparticulates (2.06 %), indicating that the incomplete adhesion between coat and core was not linked to the calendering step in the process, but originated during the co-extrudate formation. These air pockets could become entrapped between

coat and core layer when both extrusion flows merge, or could be due to different shrinking characteristics of both polymer layers upon cooling. The air gap between the coating and the core is unlikely to have any influence on the release characteristics for a tablet with an immediate release coat (as manufactured in this study) since the coat will rapidly dissolve and expose the core to the dissolution medium. However, the results show that calendering as a post-processing step for co-extruded formulations needs further optimization for drug delivery systems where the coat controls the release of the core, as the differences in thickness of the coating layer and incomplete adhesion between both layers observed in this study would induce significant variability in the release rate. Moreover, for those types of systems it will be challenging to obtain a coat that completely seals the core, a feat that is difficult to achieve with the calendering set-up used in this study, especially at the edges of the calendered tablets (Fig. 3).

Physicochemical characterisation of MPT and HCT in core and coat, respectively, was performed in order to evaluate the effect of calendering on the physical state of the incorporated drug substances. The solubility of HCT in the PEO/PEG carrier was determined by monitoring the shift in *Tg* after annealing a supersaturated physical mixture at different temperatures and subsequent quenching. First of all the Gordon-Taylor curve was established for different mixtures of HCT in the PEO/PEG carrier. The composition dependence of the glass transition temperature was fitted by the usual Gordon-Taylor law [19, 20]:

$$Tg(X_{(HCT)}) = \frac{\left[(X_{(HCT)}.Tg_{(HCT)}) + (K(1-X_{(HCT)})Tg_{(carrier)})\right]}{\left[X_{(HCT)} + K(1-X_{(HCT)})\right]} \qquad (1)$$

In equation 1 $Tg_{(HCT)}$ and $Tg_{(carrier)}$ are respectively the glass transition temperature of pure HCT and the carrier, $X_{(HCT)}$ is the HCT fraction in the mixture, and K is a fitting parameter characterizing the curvature of the evolution.

In order to determine the drug concentration dissolved in the polymer carrier at each annealing temperature the *Tg* values from the annealing experiment were plotted on the Gordon-Taylor curve. In this way the solubility curve was determined (Fig. 4). The concentration of HCT (5.6 % w/w) in the coat layer of the tablet was far below the solubility limit (31 % w/w) of HCT in the carrier.

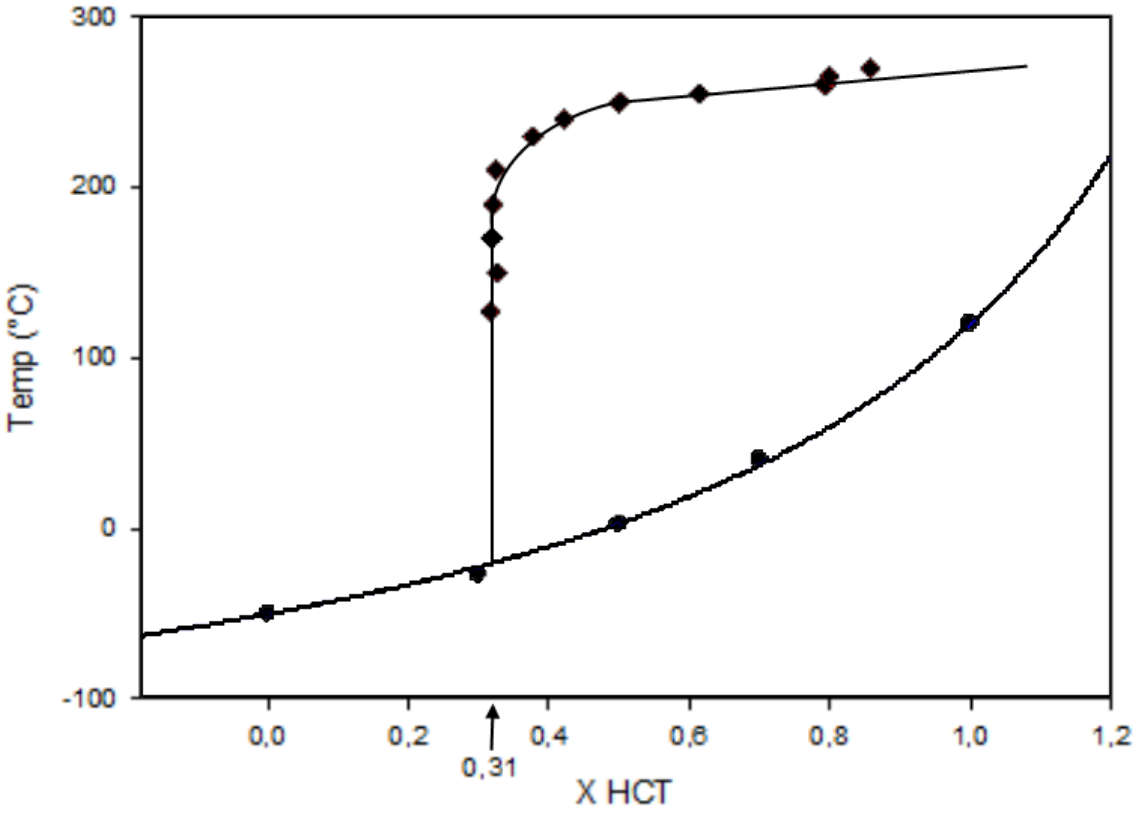

Figure 4. Evolution of glass transition temperature, fitted with a Gordon-Taylor law (●) and solubility curve (♦) for HCT in the PEO/PEG carrier.

This was confirmed by the absence of the melting endotherm of HCT in the MDSC thermogram and by the absence of any peaks representative of crystalline HCT in the X-ray diffraction pattern of the extruded coat, demonstrating HCT was present in the coat as a solid solution in the semi-crystalline polymer mixture. MDSC thermograms of MPT-loaded core formulations showed a melting peak at 118 °C. The enthalpy of fusion indicated that the main drug fraction remained crystalline in the calendered tablets: 82.3 and 85.0 % MPT was in a crystalline state in the core of formulations A and B, respectively. Similar values of MPT crystallinity were detected in the core of the mini-matrices (80.0 and 85.8 % for formulations A and B, respectively), indicating that the calendering step did not affect the solid state properties of MPT. The X-ray diffraction pattern of the core of the calendered tablet also revealed diffraction peaks of MPT, confirming that the crystalline state of MPT was at least partially maintained in the tablets. Moreover, the X-ray diffractogram did not reveal differences between the cores of calendered tablets and co-extruded mini-matrices. The pore structure of the core in both dosage forms, calendered tablets and mini-matrices, was compared using micro-CT. The percentage of internal pores was 4.00 % and 1.08 % for the core of the mini-matrices and calendered tablets, respectively. The lower amount of internal pores in the calendered tablet can be attributed to the additional densification of the material during calendering. In addition, the internal pores in the calendered tablet were smaller in size: an average structure thickness of 17 ± 20 µm, in comparison to 83 ± 5 µm for the mini-matrix (Fig. 5).

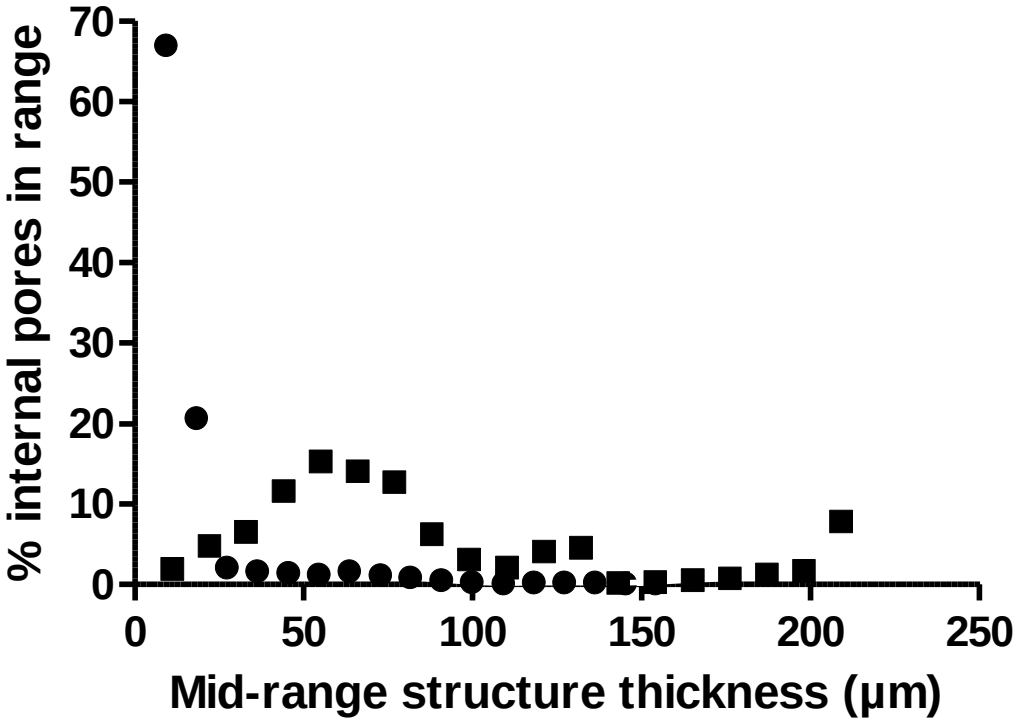

Figure 5. Pore size distributions of internal pores for mini-matrix (■) and calendered tablet (●), analyzed on a reconstructed micro-CT image.

However, these limited differences in number and size of the internal pore structure are unlikely to have an impact on drug release. The difference in sustained release of MPT between the monolithic calendered tablets and the multiparticulates is illustrated in Fig. 6.

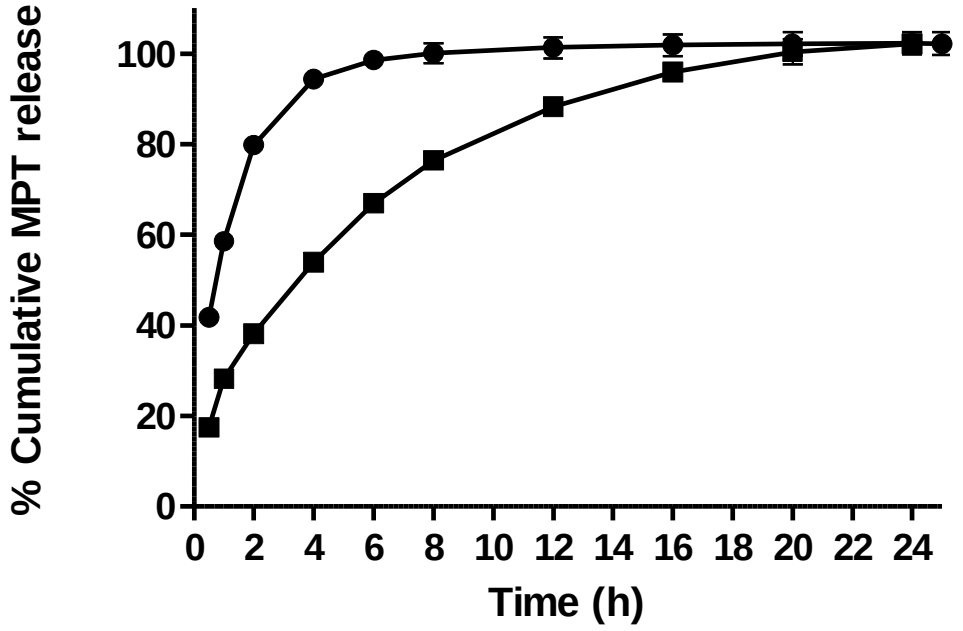

Figure 6.a. *In vitro* MPT release (in phosphate buffer pH 6.8) from mini-matrices (●) and calendered tablet (■) for formulation A. Mean (n = 3) dissolution profiles (± SD) of co-extrudates.

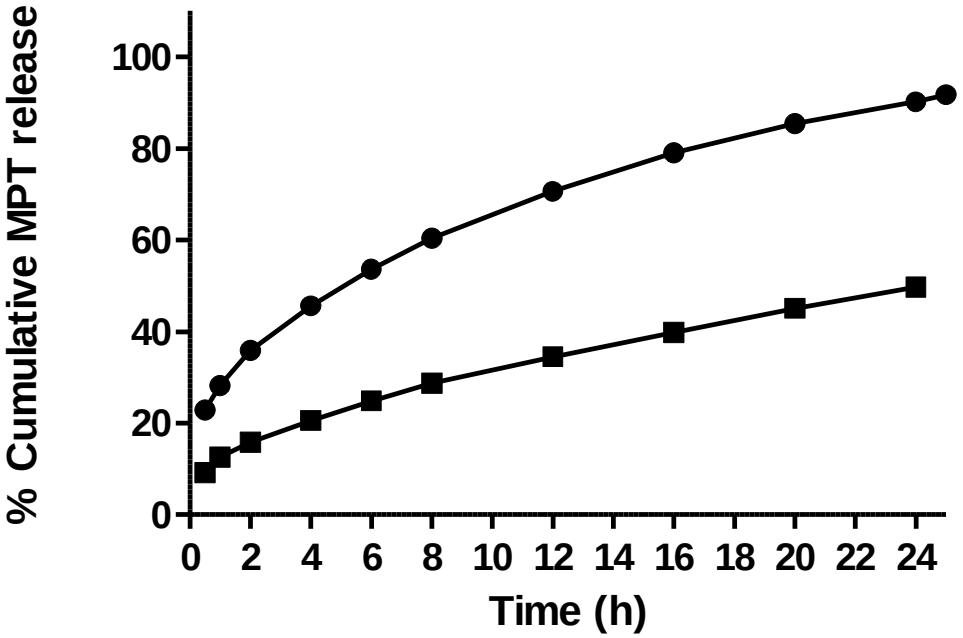

Figure 6.b. *In vitro* MPT release (in phosphate buffer pH 6.8) from mini-matrices (●) and calendered tablet (■) for formulation B. Mean (n = 3) dissolution profiles (± SD) of co-extrudates.

The MPT burst release was reduced by half for all calendered formulations in comparison with the mini-matrices. Moreover, the monolithic calendered tablet sustained MPT release to a larger extent than the mini-matrices, with a complete release after only 24 h instead of 8 h in case of formulation A. The lower mass transport rates from the calendered tablets were linked to the dimensions of the dosage forms: the core of the calendered tablet had a diameter of 7 mm and a thickness of 4 mm, whereas the core of the mini-matrices had a diameter of 3 mm and a length of 2 mm. The importance of relative surface area available highlighted the importance of diffusion path length for MPT dissolution from the matrices and was confirmed by performing dissolution tests on cylindrical extrudates with a similar surface area/volume ratio to the calendered tablet and on a central part of the calendered tablet with the same dimensions as the mini-matrices. Both test set-ups indicated that the diffusion path length is the main contributor for the differences in release profiles observed between the calendered tablets and the multiparticulate formulation. Based on the release data it is evident that manufacturing an easily swallowable tablet-shaped monolithic dosage form offered an advantage over the multiparticulate formulation for sustained drug release. For the calendered formulation B, with 5 % PEO and 30 % drug content, MPT release after 48 h was only 75 %. In contrast, complete drug release from formulation C (containing 20 % PEO and 15 % MPT) was obtained after 48 h (Fig. 7a). These differences in release profiles indicated that PEO was the main contributor for drug release. Because of the smaller dimensions of the mini-matrices, the matrix effect was of lesser importance for MPT release from the multiparticulates (Fig. 7b). The type of dosage form did not influence the immediate release profile of HCT, with a complete release within 45 min for all formulations.

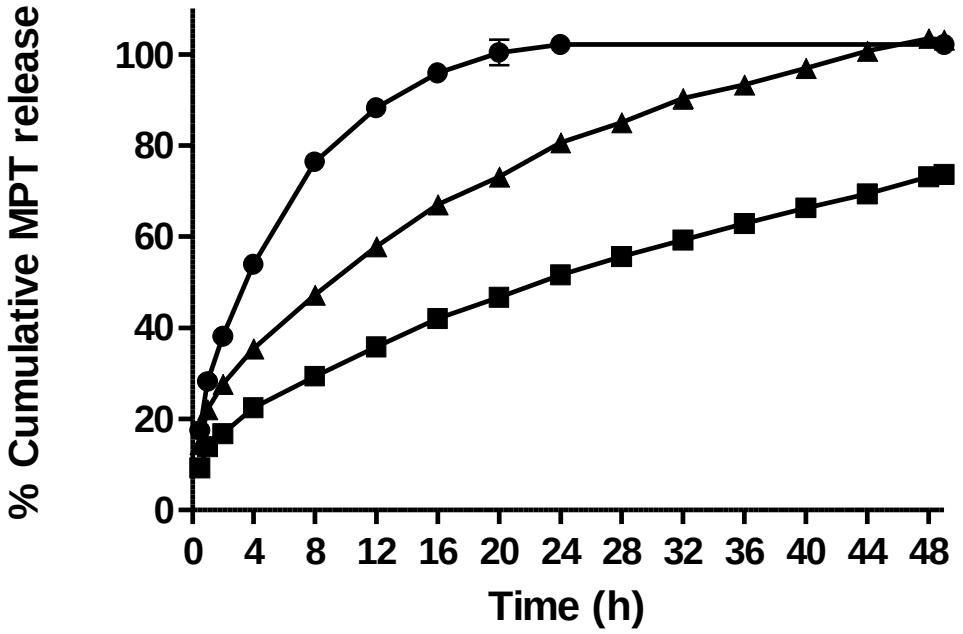

Figure 7.a. *In vitro* MPT release (in phosphate buffer pH 6.8) from calendered tablets for formulation A (●), formulation B (■), formulation C (▲). Mean (n = 3) dissolution profiles (± SD) of co-extrudates.

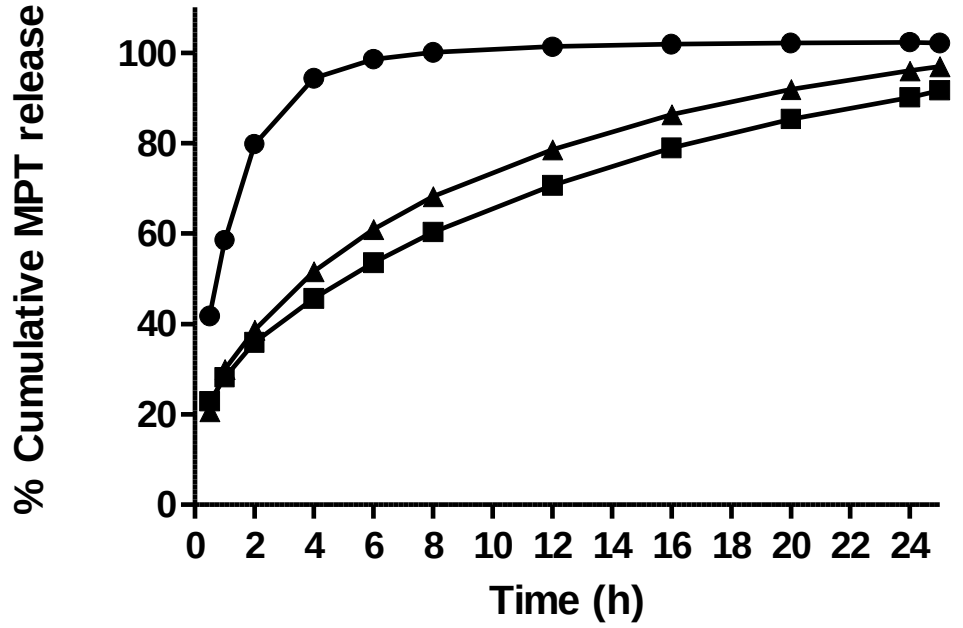

Figure 7.b. *In vitro* MPT release (in phosphate buffer pH 6.8) from mini-matrices for formulation A (●), formulation B (■), formulation C (▲). Mean (n = 3) dissolution profiles (± SD) of co-extrudates.

To assess the impact of cooling during calendering (essential to avoid sticking of the dosage form to the rolls), the effect of the cooling rate on MPT release from the calendered tablets was determined. Cooling was performed via quench-cooling in liquid nitrogen or via cooling at room temperature. However, the MPT release profiles were independent of the cooling technique. Moreover, X-ray diffractograms of the extrudates of formulations A and B demonstrated that cooling rate did not affect crystallinity of MPT in these formulations (Fig. 8).

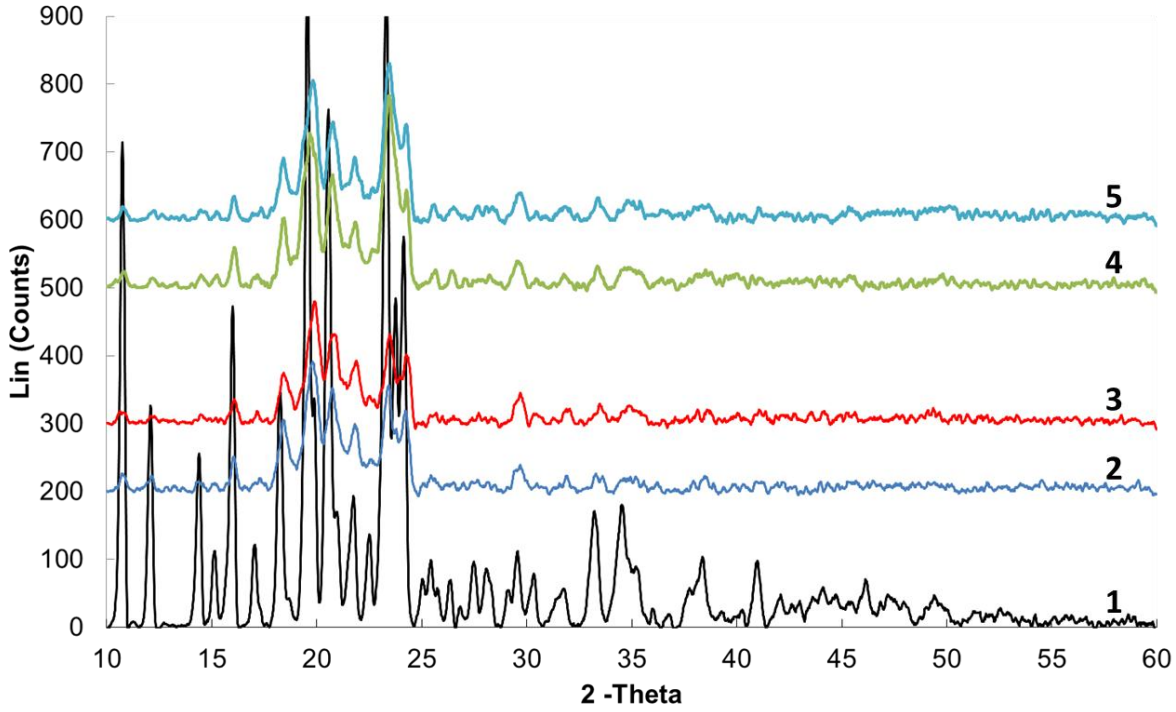

Figure 8. X-ray diffraction patterns of MPT (1), extruded core formulation B cooled at room temperature (2) or quench-cooled in liquid nitrogen (3), extruded core formulation A cooled at room temperature (4) or quench-cooled in liquid nitrogen (5).

CONCLUSION

In this study we have demonstrated that calendering is a promising downstream processing step to continuously produce tablet-shaped monolithic FDC dosage forms from multilayered matrix co-extrudates. With the calendered tablet an *in vitro* MPT release was sustained over 24 to 48 h, in combination with an immediate HCT release from the coat. The differences in diffusion path length of the final monolithic tablet-shaped dosage form mainly determined the MPT release from the core. Calendering and cooling did not affect the sustained MPT release profiles. A limited reduction of the porosity of the core after calendering indicated some additional densification of the material during calendering. The shaping technique did not alter the physicochemical state of the drugs. Further characterization using TPI revealed a gradient in coat thickness and incomplete adhesion between core and coat, the latter being inherent to the co-extrudate and independent of the calendering step, as visualized by micro-CT.

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CHAPTER 3

CO-EXTRUSION AS A PROCESSING TECHNIQUE TO MANUFACTURE A DUAL SUSTAINED RELEASE FIXED-DOSE COMBINATION PRODUCT

Parts of this chapter were submitted for publication in:

A.-K. Vynckier, J. Voorspoels, J.P. Remon, C. Vervaet. Co-extrusion as a processing technique to manufacture a dual sustained release fixed-dose combination product. Submitted to Journal of Pharmacy and Pharmacology (2015).

ABSTRACT

This study aimed to design a fixed-dose combination dosage form which provides a sustained release profile for both the freely water-soluble metformin HCl and the poorly soluble gliclazide, two anti-diabetic compounds used to treat diabetes mellitus, using co-extrusion as manufacturing technique. Developing a matrix formulation that sustained metformin release is challenging, given that its high dose requires a high drug load in the formulation and that the drug is freely soluble. From this study it was clear that co-extrusion of a coat layer, containing at least 30 % CAPA® 6506 as a hydrophobic polymer, was necessary to adequately sustain the release of the highly dosed freely soluble drug from the 70 % metformin HCl-loaded CAPA® 6506 core of the co-extrudate. To obtain a complete release over 24 h for gliclazide solubilization in Kollidon® VA, added as a second polymer to the CAPA® 6506 in the coat, was needed. In this way both active pharmaceutical ingredients (API's), which have different physicochemical characteristics, were formulated in a single dosage form, which is composed of two separate layers, each demonstrating adequate properties for the incorporated API.

CHAPTER 3

CO-EXTRUSION AS A PROCESSING TECHNIQUE TO MANUFACTURE A DUAL SUSTAINED RELEASE FIXED-DOSE COMBINATION PRODUCT

INTRODUCTION

Hot-melt extrusion (HME) has found its way to pharmaceutical industry for the production of drug-loaded matrix formulations because of its advantages over conventional techniques, such as the possibility to improve drug solubility or sustain drug release via a continuous production process. HME has been used to produce medical devices for several decades and is now being used in pharmaceutical industry for the development of drug delivery systems like transdermal patches and solid dosage forms. Co-extrusion is defined as the simultaneous extrusion of two or more materials creating a multilayered extrudate [1]. This highly efficient continuous manufacturing technique is designed to produce fixed-dose combination products but still has to find its way into pharmaceutical production. Dierickx et al. used co-extrusion to produce multilayered mini-matrices for oral drug delivery [2]. Co-extrusion has more recently been used to produce fixed-dose combination mini-matrices with a matrix core offering a range of controlled release profiles and an immediate release

coat [3], and to produce a solid dosage form which provides dual release of a single drug [4]. The aim of this study was to manufacture a dosage form with a drug-loaded coat, sustaining not only the release of the poorly soluble drug incorporated in the coat but also that of a high-dosed freely water-soluble drug incorporated in the core - using co-extrusion as a continuous production process.

The freely water-soluble metformin HCl and the poorly soluble gliclazide were used as model compounds for the development of a co-extruded concentric core/coat fixed-dose combination (FDC) product. Both are anti-diabetic compounds used to treat diabetes mellitus type 2, a complex endocrine and metabolic disorder with increasing prevalence [5]. In the treatment of patients with type 2 diabetes mellitus it has been demonstrated that combination therapy is more likely to achieve glucose control than monotherapy [6]. The most commonly used pharmacological agent as a first-line treatment in newly diagnosed patients is metformin. Sulphonylurea agents, such as gliclazide, are the most frequently used add-on treatment [7]. The combination of metformin with sulfonylurea agents such as gliclazide is commonly used and has proven to bring additive effects and an acceptable side effect profile [8, 9]. A fixed-dose combination product of metformin and gliclazide has proven to be beneficial since it can improve convenience and adherence to the prescribed therapy and to contribute to better blood glucose control [10].

Formulating metformin as a sustained release dosage form is beneficial to address issues of gastrointestinal (GI) intolerability, with up to 25 % of the patients describing some degree of GI upset leading to cessation of the therapy in 5 - 10 % of patients [11], and to avoid multiple daily dosing leading to reduced adherence [12]. It has been shown that patients that were switched from immediate release (IR) metformin to a once-daily extended release (ER) metformin formulation (Glucophage® ER) experienced fewer GI side effects although

the mean daily metformin dose was similar [13, 14]. Gliclazide modified release was previously formulated in a hypromellose-based matrix and 30 mg given once-daily showed similar efficacy and tolerability to gliclazide immediate release 80 mg/day [15]. A progressive release of the short-acting gliclazide can maintain therapeutic levels throughout the day [16]. Therefore this study aimed to design a dosage form which provides a sustained release profile for both compounds. Using co-extrusion as manufacturing technique both API's, which have different physicochemical characteristics, are formulated in a single dosage form, since the co-extruded material was composed of two separate layers, each demonstrating adequate properties for sustained release of the incorporated API.

MATERIALS AND METHODS

Materials

Metformin HCl (Fagron, Waregem, Belgium) was used as a high dose freely water-soluble drug in combination with gliclazide (Eurolabs limited, Congleton, UK). Metformin HCl and gliclazide were incorporated in the core and coat, respectively, of the co-extruded dosage form. The following materials were evaluated as excipients for the core matrix formulation: polycaprolactone with different MW: 50000 g/mol (CAPA® 6506, Perstorp, Warrington, UK), 80000 g/mol (CAPA® 6800, Perstorp, Warrington, UK) and 124000 g/mol (Purasorb® PC12, Corbion Purac Biomaterials, Gorinchem, The Netherlands), ethylcellulose (Ethocel® std 10, Colorcon, Dartford Kent, United Kingdom), dibutyl sebacate (Sigma-Aldrich, Bornem, Belgium), polyethylene glycol 4K (PEG 4K, MW: 4000 g/mol, Fagron, Waregem, Belgium), poloxamer 188 (Lutrol® F68, BASF, Ludwigshafen, Germany), a 8:2 blend of polyvinyl acetate and polyvinylpyrrolidone (Kollidon® SR, BASF, Ludwigshafen, Germany), low-density polyethylene (LDPE, SABIC, Riyadh, Saudi Arabia). For the coat formulation polycaprolactone with a MW of 50000 g/mol (CAPA® 6506, Perstorp, Warrington, UK) and copovidone (Kollidon® VA 64, BASF, Ludwigshafen, Germany) were used as excipients. All other chemicals used were of analytical grade.

Hot-melt extrusion and co-extrusion

In a first phase hot-melt extrusion was performed to select an appropriate polymer matrix for the highly metformin HCl-loaded core extrudate, using a co-rotating Prism Eurolab 16 mm fully intermeshing twin screw extruder (ThermoFisher Scientific, Karlsruhe, Germany)

connected to a cylindrical die having a diameter of 4 mm (Guill, West Warwick, USA). For the core formulations the processing temperatures are given in Table 1. Premixes of drug, polymer and additives were fed into the extruder using a Brabender Flexwall® loss-in-weight powder feeder (Brabender, Duisburg, Germany) at a feed rate of 300 g/h for the core material. A screw speed of 20 rpm was used for the extruder. To compare release from core extrudates formulated with different polymer matrices the extrudate was manually cut in mini-matrices of 2 mm length.

In a second phase co-extrusion was carried out using two co-rotating Prism Eurolab 16 mm twin screw extruders (ThermoFisher Scientific, Karlsruhe, Germany), both connected to the co-extrusion die (Guill, West Warwick, USA). A cylindrical co-extrudate with a core diameter of 4 mm and a concentric coat with a thickness of 1 mm (total co-extrudate diameter: 6 mm) was manufactured. The zones of the barrel of the extruders producing the core and the coat were heated from feed opening to die-end to 90/100/120/110/110/110 and 100/110/110/110/110/120 °C, respectively. The co-extrusion die was heated to 130 °C. The core and coat premix were fed separately into an extruder using a Brabender Flexwall® loss-in-weight powder feeder (Brabender, Duisburg, Germany) at a feed rate of 250 g/h for the coat and 300 g/h for the core material. A screw speed of 20 rpm was used for both extruders. After cooling the cylindrical co-extrudate was manually cut into tablets of 5 or 10 mm length.

In vitro drug release

In vitro dissolution was performed using United States Pharmacopeia (USP) dissolution apparatus 2 (paddles) on an Evolution 6300 dissolution system (Distek, New Brunswick, New Jersey, USA), coupled with an Evolution 4300 automatic dissolution sampler (Distek, New

Brunswick, New Jersey, USA). The temperature of the dissolution medium (900 ml) was kept at 37 ± 0.5 °C and the rotational speed of the paddles was set to 100 rpm. Phosphate buffer pH 6.8 or pH 7.4 (USP) was used as the dissolution medium. Samples (filtered using Distek 45 µm filters) of 5 ml were withdrawn at 0.5, 1, 2, 4, 6, 8, 12, 16, 20 and 24 hours for the determination of the concentration of both compounds in the dissolution medium. For the initial experiments with the coat formulation an acetate buffer pH 4.1 was used since this is a more discriminative pH to test the solubility improvement of gliclazide, which has a pH-dependent solubility [17]. In these experiments samples (filtered using Distek 45 µm filters) of 5 ml were withdrawn at 5, 10, 20, 30, 45, 60 and 120 min. Samples were analyzed spectrophotometrically at 232.8 nm and 226.4 nm, using a UV-spectrophotometer (UV-1800, Shimadzu, Deurne, Belgium) and applying an appropriate calibration curve for quantification of metformin and gliclazide, respectively. Each experiment was performed in triplicate.

Modulated differential scanning calorimetry

The crystallinity of the drug in the matrices and the thermal behavior of pure compounds, physical mixtures and corresponding extrudates were studied using a differential scanning calorimeter Q2000 V24.8 equipped with a refrigerated cooling system (TA Instruments, Leatherhead, UK). Nitrogen was used as purge gas through the DSC cell (50 ml/min) and the refrigerated cooling system (RCS) unit (300 ml/min). Samples (± 5 mg) were run in hermetically closed Tzero pans with perforated lid, supplied by TA Instruments, with an underlying heating rate of 2 °C/min. The modulation period and amplitude were set at 60 s and 0.318 °C, respectively. Mass of sample pan and empty reference pan were taken into account. Temperature and enthalpy calibration was performed with an indium standard,

whereas calibration of the heat capacity was performed using a sapphire standard. Modulated differential scanning calorimetry (MDSC) data were analyzed using the TA instruments Universal Analysis 2000 V4.7A software. Melting enthalpies were determined in the total heat flow signal. Melting temperatures were reported as peak temperatures. Degree of crystallinity was calculated based on melting enthalpy of the drug in the extruded formulation compared to the pure drug.

X-ray diffraction

Crystallinity was analyzed using X-ray diffraction (XRD) on pure compounds, physical mixtures and corresponding extrudates. X-ray diffraction was performed on a D5000 diffractometer with Cu Kα radiation (λ = 1.54 Å) (Siemens, Karlsruhe, Germany) and a voltage of 40 mV in the angular range (2θ) varying from 10 to 60 ° using a step scan mode with a step size of 0.02 ° and a measuring time of 1 s/step.

Adhesion

The adhesion between core and coat was analyzed using a tensile tester with a load cell capacity of 100 N (LF Plus, Lloyd Instruments, West Sussex, UK). Co-extruded mini-matrices of 2 mm in length were placed on a metal disk with a central opening of 4.2 mm, above which the core of the co-extruded mini-matrices was positioned to make sure that only the coat was supported by the device. A probe with a diameter of 2 mm was used to apply a downward force on the core (preload 1 N; extension rate 100 mm/min) and the maximum force needed to separate coat from core was measured. The test was repeated 10 times, mean result and standard deviation are reported.

RESULTS AND DISCUSSION

Developing a matrix formulation that sustained metformin release is challenging, given that its high dose requires a high drug load in the formulation and that the drug is freely soluble. In order to obtain a sustained release profile for metformin, several thermoplastic polymers were hot-melt extruded and evaluated for processability, macroscopic properties and *in vitro* drug release from mini-matrices with a length of 2 mm (Table 1).

Matrix formulation	Metformin HCl load	Extrusion temperature (°C) From feed opening to die-end	Torque *	Macroscopic properties	Duration (h) to 100 % release
EC + 33% DBS	60 %	100/120/120/120/120/120/120	24 %	Moderate	12 h
EC + 33% DBS	70 %	100/150/150/150/150/150/150	20 %	Inacceptable	/
Kollidon® SR	50 %	150/170/170/170/170/150/150	90 %	Inacceptable	/
Kollidon® SR + 10% Lutrol® F68	70 %	150/175/175/175/175/150/150	67 %	Good	4 h
Kollidon® SR + 20% Lutrol® F68	70 %	140/165/165/165/165/140/140	65 %	Good	2 h
LDPE	70 %	100/100/100/100/100/100/100	25 %	Good	2 h
LDPE + 10% PEG 4K	70 %	110/110/110/110/110/110/110	15 %	Good	2 h
LDPE + 20% PEG 4K	70 %	95/95/95/95/95/95/95	30 %	Good	2 h
CAPA® 6506	60 %	60/60/60/60/60/60/60	89 %	Good	4 h
CAPA® 6506	70 %	80/80/80/80/80/80/80	75 %	Good	/

*Torque in % of max value (12Nm/shaft).

Table 1. Evaluation of processability, macroscopic properties and *in vitro* drug release from mini-matrices (length 2 mm) during a polymer screening of metformin HCl-loaded core formulations.

Ethylcellulose (EC) could efficiently sustain metformin release over 12 h from EC-based formulations containing 60 % metformin HCl. However, at the required extrusion conditions (120 and 150 °C for a 60 and 70 % metformin HCl formulation, respectively) extrudates with a highly irregular shape which easily pulverized were formed, even as EC was combined with dibutyl sebacate (DBS) as plasticizer (EC : DBS ratio of 2:1). The higher extrusion temperature even resulted in a brown discoloration of the extruded EC-based samples. Using Kollidon® SR as matrix former resulted in a too high torque, even at a process temperature of 170 °C for a 50 % metformin HCl formulation. Inclusion of Lutrol® F68

improved the processability and the appearance of the metformin/Kollidon® SR extrudates: the addition of 10 and 20 % Lutrol® F68 as a plasticizer allowed to increase drug load to 70 %, yielding smooth extrudates at a process temperature of 175 and 165 °C, respectively. However, the sustained release capacity of these formulations was limited: complete drug release after 4 and 2 h from formulations with a plasticizer content of 10 and 20 %, respectively. Similarly sustained metformin release from an LDPE matrix was limited at a 70 % drug content: complete release after 2 h. In addition, the surface of these extrudates was irregular, even after the addition of PEG 4K as plasiticizer. Using CAPA® 6506 as a matrix polymer, mixtures with 60 % metformin HCl could be processed at a temperature of 60 °C, yielding solid extrudates with a regular shape and smooth surface. Even at a drug load of 70 % the CAPA® 6506 matrix could be processed via hot-melt extrusion at a temperature of only 80 °C. Since these CAPA® 6506 matrices had a good appearance and could be manufactured at a low temperature, polycaprolactone was selected for further use. A metformin HCl content of 70 % was the maximum drug load possible: since 90 % of the drug remained crystalline, higher drug loadings were impossible to process into high-quality core extrudates. Subsequently the effect of polycaprolactone grades with different molecular weight on the release profile was tested (all formulations were processed at a temperature of 100 °C in all zones of the extruder). The entire drug content was released after 4 h from a 60 % metformin HCl extrudate formulated with CAPA® 6506, while polycaprolactones with a higher molecular weight could sustain metformin release over 8 h. None of the polycaprolactone grades could sustain the metformin release from a highly drug-loaded extrudate over 24 h.

Since a drug load of at least 60 % was essential to obtain a viable dosage form, a layer of CAPA® 6506, with a thickness of 1 mm, was applied around the core via a co-extrusion

process to reduce the metformin release rate. This strategy allowed to sustain drug release over a longer period, even a reduction of the length of the dosage form from 10 to 5 mm was needed to release the entire drug fraction within a 24 h period (Fig. 1).

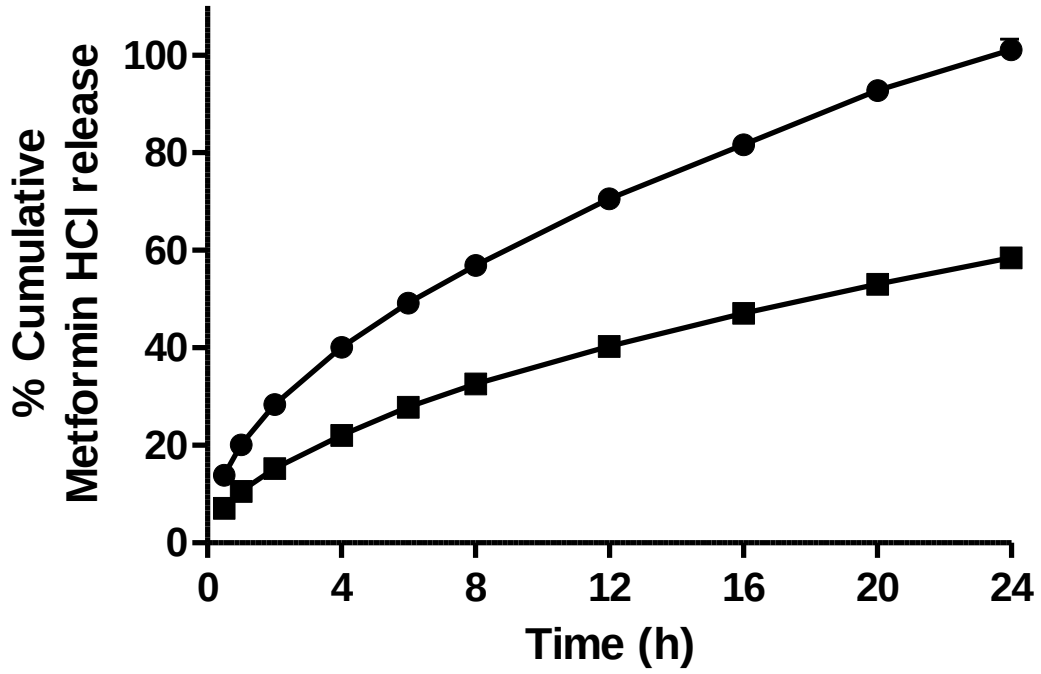

Figure 1. Influence of the length of the co-extruded dosage forms on *in vitro* metformin HCl release: 5 (●) and 10 mm (■). The CAPA® 6506 core of the co-extruded dosage form contained 60 % metformin HCl, while the coat consisted of pure CAPA® 6506. Mean (n=3) dissolution profiles (± SD), dissolution at 37 °C and 100 rpm in phosphate buffer pH 6.8.

In addition to sustaining the release of metformin from the core, the coat layer also needed to sustain the release of gliclazide, the drug incorporated in the coat. When formulating 1.2 % gliclazide in CAPA® 6506 only 29 % of the gliclazide content was released after 24 h in a pH 4.1 acetate buffer. Hence solubilization of the drug was indispensable to achieve complete gliclazide release. Since the potential of Kollidon® VA 64 to improve the solubility of poorly water-soluble drugs by manufacturing a solid dispersion via hot-melt extrusion has previously been shown [18, 19], mixtures of 1.2 and 10 % gliclazide in Kollidon® VA 64 were

hot-melt extruded. Both formulations yielded a transparent extrudate and the *in vitro* gliclazide release in pH 4.1 acetate buffer was complete after 20 min. This indicated that Kollidon® VA 64 was an efficient solubilizer for gliclazide in the CAPA® 6506 coat formulation, the latter polymer being an essential ingredient to sustain metformin release from the core. When combining CAPA® 6506 with Kollidon® VA 64 via hot-melt extrusion, both polymers formed a separate phase in the extrudate as thermal analysis revealed a CAPA® 6506 melting endotherm at 57.4 °C and a glass transition of Kollidon® VA 64 at 108.0 °C. Gliclazide release from a coat formulation containing 5 % drug depended on the Kollidon® VA 64 : CAPA® 6506 ratio, a 55:40 ratio providing complete and sustained drug release over 24 h (Fig. 2).

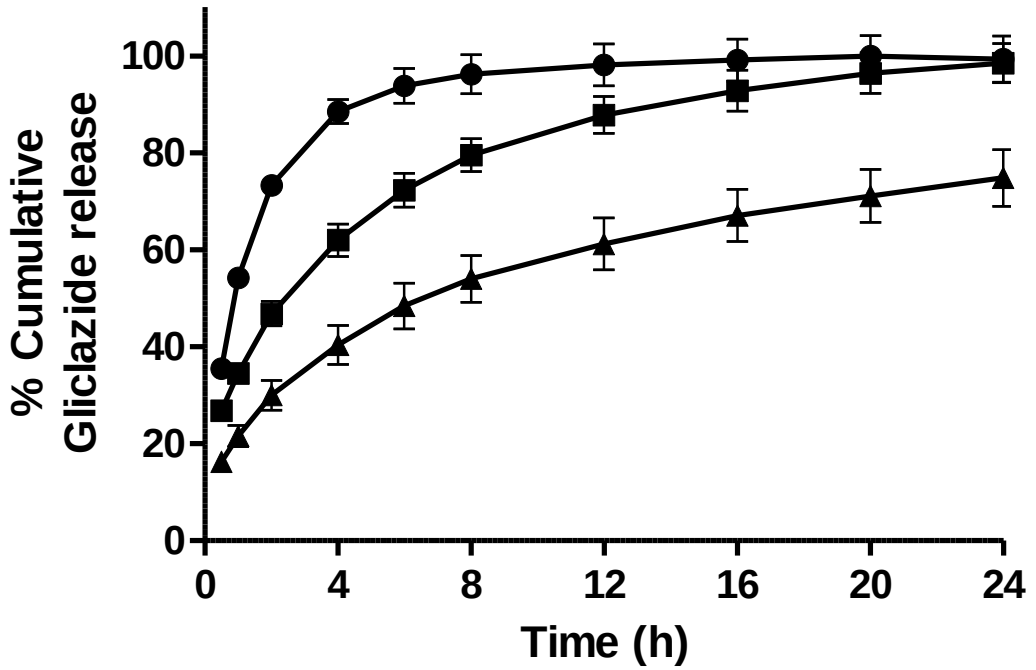

Figure 2. Influence of Kollidon® VA 64 : CAPA® 6506 ratio on the *in vitro* gliclazide release from the coat of co-extruded dosage forms: 65:30 (●), 55:40 (■) and 45:50 (▲) Kollidon® VA 64 : CAPA® 6506 ratio. The co-extruded dosage form contained 5 % gliclazide in its coat, while the core consisted of pure CAPA® 6506. Mean (n=3) dissolution profiles (± SD), dissolution at 37 °C and 100 rpm in phosphate buffer pH 6.8.

The *in vitro* metformin release profiles from co-extrudates with a 70 % metformin HCl-loaded CAPA® 6506 core and a coat with different Kollidon® VA 64 : CAPA® 6506 ratios (ranging from 60:40 to 80:20) indicated that a CAPA® 6506 concentration of at least 30 % was needed in the coat to sufficiently sustain metformin release over 24 h from the core of the co-extrudate (Fig. 3). Based on these results a Kollidon® VA 64 : CAPA® 6506 ratio of 55:40 was selected as matrix composition for the coat layer in the co-extruded formulation.

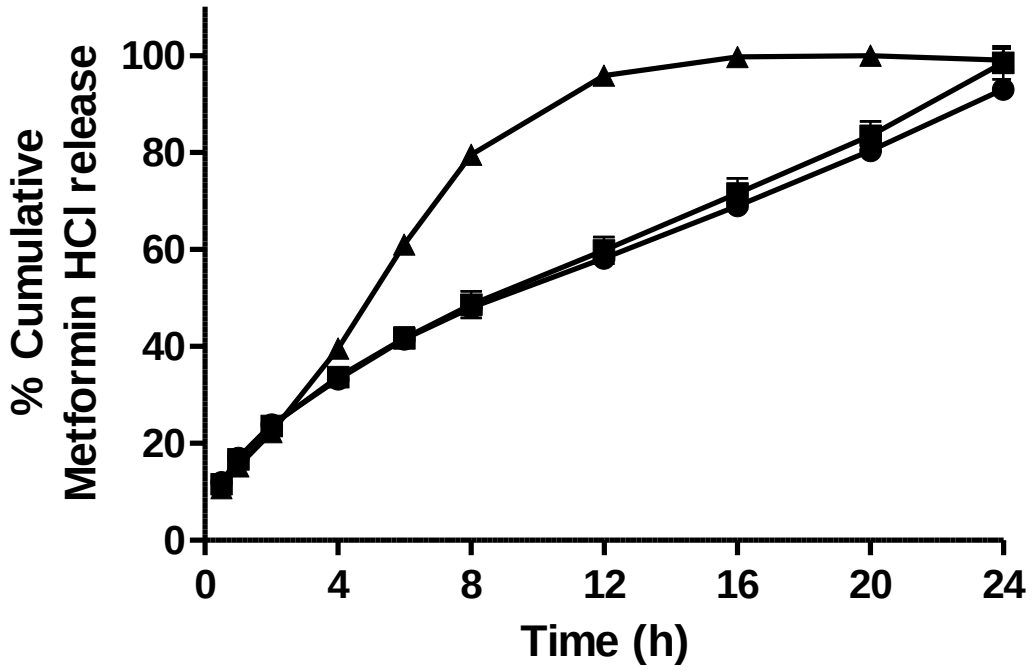

Figure 3. Influence of Kollidon® VA 64 : CAPA® 6506 ratio on the *in vitro* metformin HCl release from the core of co-extruded dosage forms: 60:40 (●), 70:30 (■) and 80:20 (▲) Kollidon® VA 64 : CAPA® 6506 ratio. The co-extruded dosage form contained 70 % metformin HCl in its CAPA® 6506 core, combined with a placebo Kollidon® VA 64 and CAPA® 6506 coat. Mean (n=3) dissolution profiles (± SD), dissolution at 37 °C and 100 rpm in phosphate buffer pH 6.8.

For the final co-extruded dosage form, having a metformin HCl load of 70 % in the core and a CAPA® 6506 concentration of 40 % in the coat, metformin release from the core was

following zero-order kinetics. Gliclazide release from the coat was sustained over 24 h with a substantial burst release (25.5 % after 0.5 h) (Fig. 4).

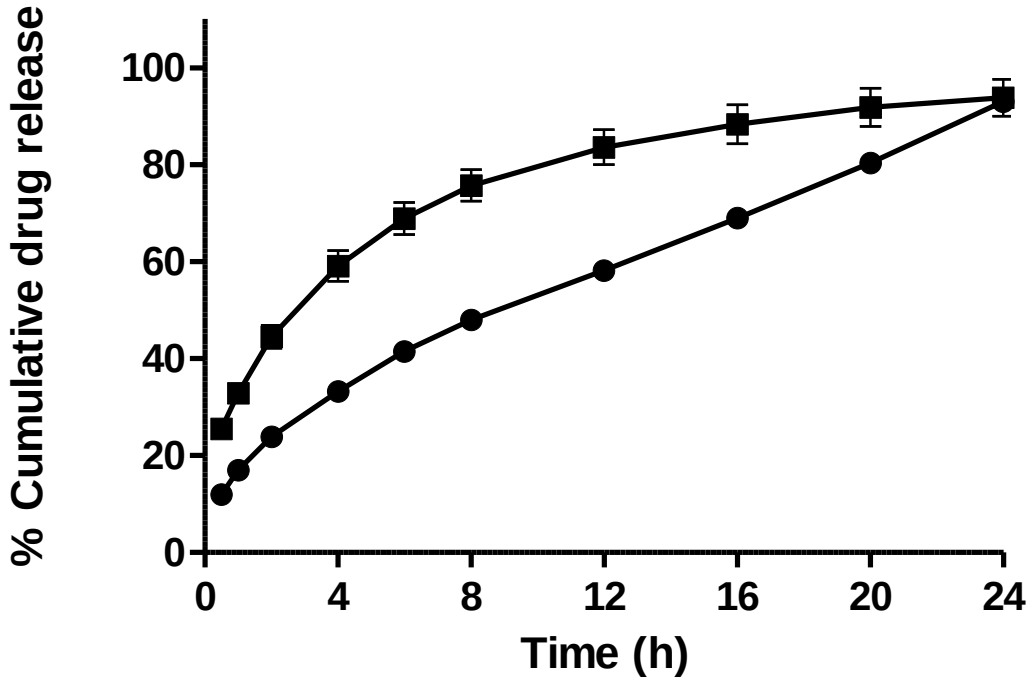

Figure 4. *In vitro* metformin HCl (●) and gliclazide (■) release from co-extruded dosage forms. The core of the co-extruded dosage form contained 70 % metformin HCl + 30 % CAPA® 6506, while the coat was formulated with 5 % gliclazide, 55 % Kollidon® VA 64 and 40 % CAPA® 6506. Mean (n=3) dissolution profiles (± SD), dissolution at 37 °C and 100 rpm in phosphate buffer pH 6.8.

The physicochemical state of both active compounds in the final co-extruded formulation was characterized using XRD and MDSC. The gliclazide peaks which were detected in the diffractogram of the physical mixture of the coat, were absent from the diffraction pattern of the extruded coat (Fig. 5). These results pointed out that gliclazide was present in dissolved state in the coat of the co-extrudate. MDSC analysis also indicated that gliclazide was solubilized in the amorphous Kollidon® VA 64 phase in the formulation. In contrast, after extrusion 90 % of the metformin HCl fraction (melting endotherm at 221.3 °C) remained crystalline in the CAPA® 6506 matrix (Fig. 6).

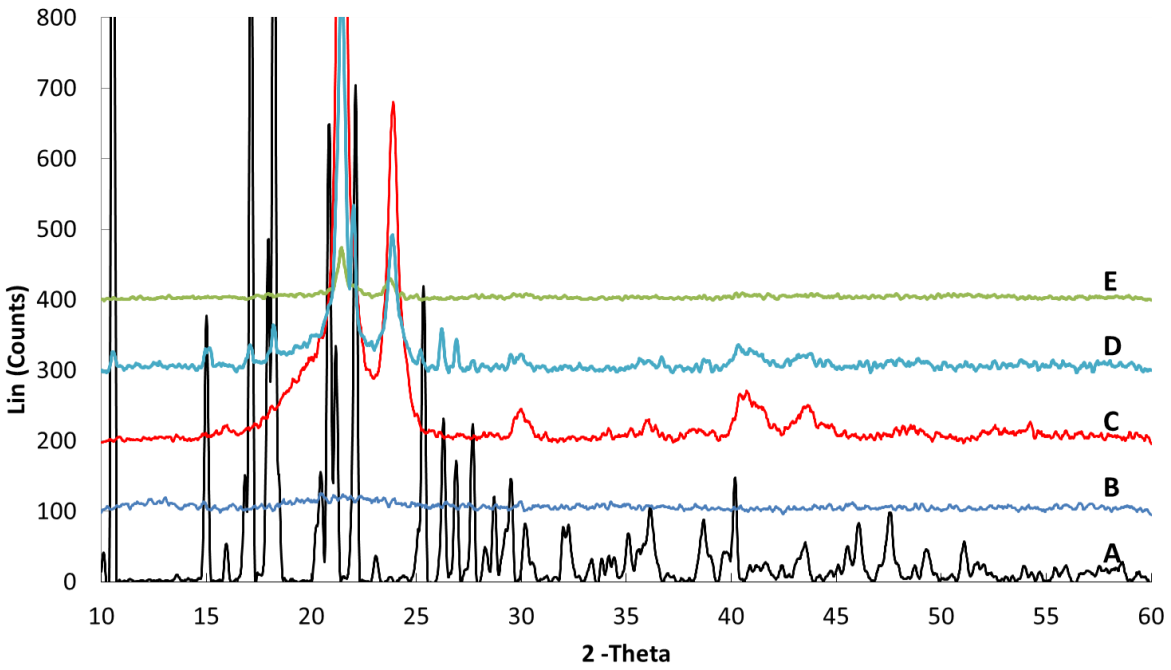

Figure 5. X-ray diffraction patterns of (from bottom to top): gliclazide (A), Kollidon® VA 64 (B), CAPA® 6506 (C), physical mixture of the coat formulation containing 5 % gliclazide (D) and extruded coat with the same composition (E).

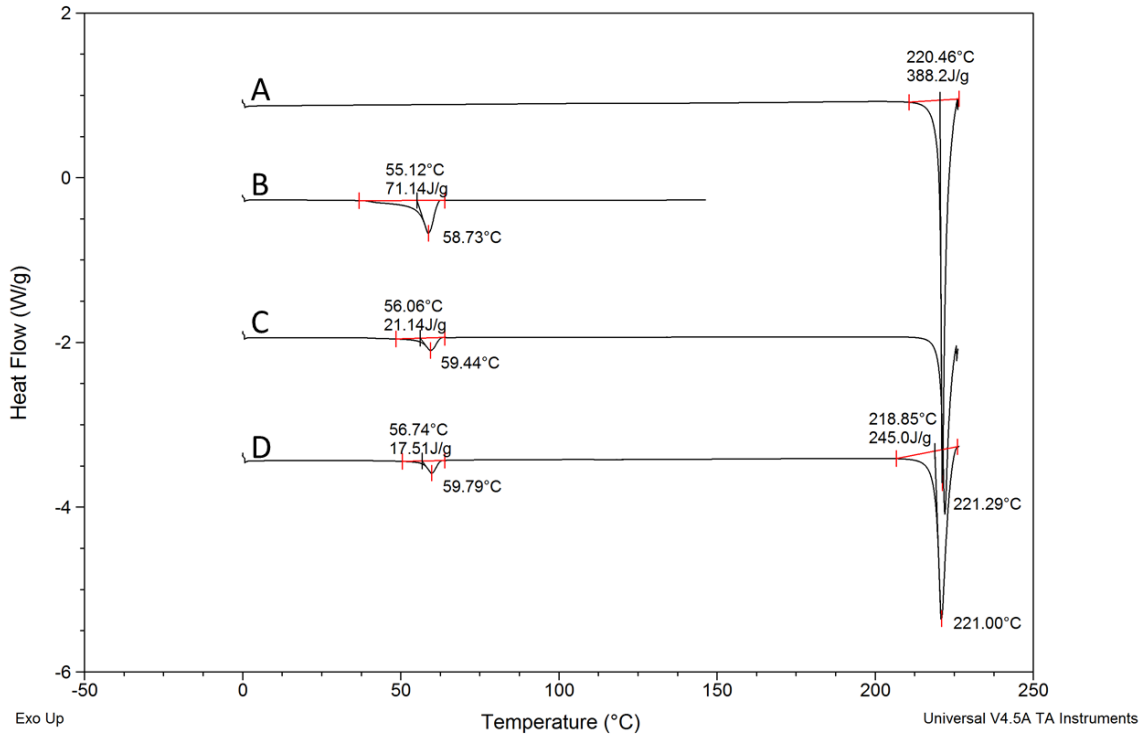

Figure 6. MDSC thermograms of (from top to bottom): metformin HCl (A), CAPA® 6506 (B), physical mixture of the core formulation containing 70 % metformin HCl and 30 % CAPA® 6506 (C) and extruded core with the same composition (D).

In order to characterize the degree of adhesion between both layers of the co-extrudate the adhesion force between core and coat was measured. For the final formulation the adhesion test revealed that the average force needed to detach the core from the coat was 65.6 ± 10.9 N. This high value can be attributed to the fact that CAPA® 6506 was used in both core and coat, ensuring a high degree of interaction between both layers.

CONCLUSION

Bilayer dosage forms containing an anti-diabetic drug in both core and coat were successfully developed by co-extrusion, offering a fixed-dose combination product sustaining the release over 24 h for both metformin HCl and gliclazide. A coat layer with at least 30 % CAPA® 6506 as a hydrophobic polymer was essential to adequately sustain metformin release from the CAPA® 6506 core of the co-extruded dosage form. Inclusion of Kollidon® VA 64 in the coat ensured complete release over 24 h of gliclazide via solubilization of the drug in the Kollidon® VA 64 phase.

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CHAPTER 4

ENTERIC PROTECTION OF NAPROXEN IN A FIXED-DOSE COMBINATION PRODUCT PRODUCED BY HOT-MELT CO-EXTRUSION

Parts of this chapter are published in:

A.-K. Vynckier, M. De Beer, T. Monteyne, J. Voorspoels, T. De Beer, J.P. Remon, C. Vervaet. Enteric protection of naproxen in a fixed-dose combination product produced by hot-melt co-extrusion. International Journal of Pharmaceutics, doi: 10.1016/j.ijpharm.2015.06.010 (2015).

ABSTRACT

In this study hot-melt co-extrusion is used as a processing technique to manufacture a fixed-dose combination product providing enteric protection to naproxen incorporated in the core and immediate release to esomeprazole magnesium embedded in the coat. Both core and coat were first independently developed. The plasticizing effect of naproxen and triethyl citrate (TEC) was tested on the enteric polymers investigated (Eudragit® L100-55, HPMC-AS-LF and HPMCP-HP-50). Core matrix formulations containing HPMC-AS-LF, TEC and a naproxen load of 15, 30 and 50 % were processed and characterized. The *in vitro* naproxen release in 0.1 N HCl was prevented for 2 h for all formulations. The physicochemical state of the drug in the extrudates was determined and a stability study was performed. Intermolecular interactions between naproxen and polymer were identified using attenuated total reflection Fourier-transform infrared (ATR FT-IR) spectroscopy. Esomeprazole magnesium was formulated in an immediate release polymer, separated from the naproxen-containing enteric layer. When esomeprazole magnesium was formulated in a polyethylene oxide 100K : polyethylene glycol 4K (1:1) matrix, the formulation could be easily processed (lower torque) and complete *in vitro* drug release was observed after 45 min. When co-extruding the core/coat dosage form it was observed that a third layer of polymer, separating the naproxen-loaded enteric formulation in the core from the coat, is required to prevent degradation of the acid-labile esomeprazole magnesium at the core/coat interface.

CHAPTER 4

ENTERIC PROTECTION OF NAPROXEN IN A FIXED-DOSE COMBINATION PRODUCT PRODUCED BY HOT-MELT CO-EXTRUSION

INTRODUCTION

Hot-melt co-extrusion is defined as the simultaneous hot-melt extrusion of two or more materials creating a multilayered extrudate [1]. This continuous manufacturing technique still has to break through in pharmaceutical production, although several literature reports are already available on the use of co-extrusion for oral drug delivery. Quintavalle et al. were the first to produce cylindrical co-extrudates with controlled drug release via hot-melt extrusion, using polyethylene glycol as hydrophilic matrix and stearic acid or microcrystalline wax as hydrophobic matrix [2, 3]. Co-extruded mini-matrices have recently been formulated using core/coat technology with drugs incorporated in different polymer matrices in order to steer the release of different drugs [4, 5] or to provide a dual release of a single drug [6]. Co-extrusion offers the potential to formulate fixed-dose combination products containing two chemically incompatible drugs in separate layers.

The present study investigated if hot-melt co-extrusion allowed to manufacture a fixed-dose combination product providing enteric protection to the active pharmaceutical ingredient (API) incorporated in the core and immediate release to the API embedded in the coat.

Several enteric polymers were tested as core matrix former in combination with naproxen. This non-steroidal anti-inflammatory drug (NSAID) was used as a model drug. Since gastro-protective co-therapy using a proton pump inhibitor is recommended to decrease the incidence of NSAID-related adverse events, esomeprazole magnesium was incorporated in the coat [7, 8]. Esomeprazole magnesium was formulated in a separate non-enteric polymer layer providing immediate drug release, which is essential to achieve rapid absorption of esomeprazole [9]. For both the core and coat layers different polymers were tested and their influence on release, physicochemical state characteristics and stability was monitored. Finally it was evaluated if co-extrusion of a core/coat dosage form allowed to formulate the two chemically incompatible API's in a fixed-dose combination that offered the desired release profile for both API's [8].

MATERIALS AND METHODS

Materials

Naproxen (pKa 4.15) (Fagron, Waregem, Belgium) and esomeprazole magnesium trihydrate (Nifty labs, Hyderabad, India) were chosen as model drugs. Vimovo® (AstraZeneca, Brussels, Belgium), containing 500 mg enteric-coated naproxen and 20 mg non-enteric-coated esomeprazole magnesium, was used as a commercially available reference. The following enteric polymers were used: methacrylic acid – ethyl acrylate copolymer (1:1) Type A (Eudragit® L100-55, Evonik, Darmstadt, Germany), hydroxypropyl methylcellulose acetate succinate (HPMC-AS-LF, Aqoat® AS-LF, Shin-Etsu, Tokyo, Japan) and hydroxypropyl methylcellulose phthalate (HPMCP-HP-50, Shin-Etsu, Tokyo, Japan). Triethyl citrate (TEC, Sigma-aldrich, Bornem, Belgium) and talc (Luzenac® Pharma, Imerys Talc, Gent, Belgium) were used as excipients in the core formulation. The polymers used in the coat formulation were polyethylene oxide 100K (PEO 100K, Mw: 100000 g/mol, SentryTM Polyox® WSR N10, Colorcon, Dartford Kent, United Kingdom), polyvinylpyrrolidone (Kollidon®12 PF, Mw: 2500 g/mol, BASF, Ludwigshafen, Germany), hydroxypropyl methylcellulose (Methocel® E3, viscosity: 3 mPa.s, Colorcon, Dartford Kent, United Kingdom), hydroxypropyl cellulose (Klucel® EF, Mw: 80000, Ashland, Covington, USA) and polyethylene glycol 4K (PEG 4K, Mw: 4000 g/mol, Fagron, Waregem, Belgium). All other chemicals were of analytical grade.

Hot-melt extrusion and co-extrusion

In a first step hot-melt extrusion was performed to select an appropriate polymer matrix for core and coat separately, using a co-rotating Prism Eurolab 16 mm fully intermeshing twin

screw extruder (ThermoFisher Scientific, Karlsruhe, Germany) connected to a co-extrusion die having a core and coat insert with a diameter of 4 and 6 mm, respectively (Guill, West Warwick, USA). For the core formulations the processing temperatures are given in Table 1.

Matrix polymer	TEC conc.*	Naproxen load	Processing temperature(°C) (from feed opening to die-end)	Appearance	Degree of crystallinity
Eudragit® L100-55	10 %	15 %	100/100/100/100/120/120/120	clear	/
HPMC-AS-LF	10 %	15 %	150/150/150/150/150/150/150	clear	/
HPMCP-HP-50	10 %	15 %	145/145/145/145/145/145/145	clear	/
Eudragit® L100-55	10 %	30 %	110/110/110/110/125/125/125	clear	1.3 %
			100/100/100/100/110/110/110	opaque	37.3 %
HPMC-AS-LF	10 %	30 %	120/120/120/120/120/120/120	clear	2.6 %
			100/100/100/100/100/100/100	opaque	29.0 %
HPMCP-HP-50	10 %	30 %	130/130/130/130/130/130/130	clear	0.7 %
			115/115/115/115/115/115/115	opaque spots	1.5 %
HPMC-AS-LF	/	50 %	120/120/120/110/110/100/100	opaque	70.8 %

*The concentration of plasticizer is expressed in relation to the matrix polymer

Table 1. Extrusion temperature, degree of crystallinity and extrudate appearance of 15, 30 and 50 % naproxen-loaded core extrudates, with different matrices.

The coat formulation was processed at a temperature of 100 °C in all zones of the extruder and the die. Premixes of drug, polymer and additives were fed into the extruder using a Brabender Flexwall® loss-in-weight powder feeder (Brabender, Duisburg, Germany) at a feed rate of 375 g/h for the coat and 300 g/h for the core material. A screw speed of 120 rpm was used for each of the extruders.

In a second phase co-extrusion was carried out using two co-rotating Prism Eurolab 16 mm twin screw extruders (ThermoFisher Scientific, Karlsruhe, Germany), both connected to the co-extrusion die (Guill, West Warwick, USA). A cylindrical co-extrudate with a core diameter of 4 mm and a concentric coat with a thickness of 1 mm (total co-extrudate diameter: 6 mm) was manufactured. After cooling to room temperature the cylindrical co-extrudate was manually cut into cylinders of 10 mm length, which were used for further analysis.

In vitro drug release

In vitro dissolution was performed using United States Pharmacopeia (USP) dissolution apparatus 2 (paddles) on an Evolution 6300 dissolution system (Distek, New Brunswick, New Jersey, USA), coupled with an Evolution 4300 automatic dissolution sampler (Distek, New Brunswick, New Jersey, USA). The temperature of the dissolution medium was kept at 37 ± 0.5 °C and the rotational speed of the paddles was set to 100 rpm. To characterize the release of naproxen 750 ml of a 0.1 N solution of HCl was used as the dissolution medium for the first 2 h. After collecting the 2 h sample, 250 ml $Na_3PO_4.12H_2O$ 0.2 M was added to the dissolution vessel to adjust the pH of the medium to 6.8. Samples (filtered using Distek 45 µm filters) of 5 ml were withdrawn after 0.5, 1, 1.5 and 2 h in the acid stage and consequently after 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h and 10 h in the pH 6.8 buffer stage. To assess the esomeprazole magnesium release a dissolution test in demineralized water was performed for 2 h. For this *in vitro* dissolution test samples of 5 ml were withdrawn after 5, 10, 15, 20, 30, 45, 60, 90 and 120 min. Each experiment was performed in triplicate.

Ultra high performance liquid chromatography analysis

For the determination of both active compounds in the dissolution samples an ultra high performance liquid chromatography (UHPLC) analysis was performed using a reversed-phase C18 column with a gradient system (10 min) based on aqueous 10 mM ammonium acetate (A) and acetonitrile (B). The gradient used was: a linear ramp from 0 to 5 min going from 85 % A + 15 % B to 50 % A + 50 % B, changing over to 5 % A + 95 % B at 5.1 min, maintained for 1.8 min and afterwards changing over to chromatographic start conditions 85 % A + 15 % B

from 6.9 min to 7 min, followed by an equilibration of 3 min preceding the next injection. An Acquity CSH C18 column (1.7 µm particle size, 2.1 x 100 mm) (Waters, Brussels, Belgium) was used in an oven set at 40 °C. The flow rate was set at 0.35 ml/min, injection volume was 0.3 µl. A photo-diode array detector (Acquity, Waters, Brussels, Belgium) was used. For the quantification of esomeprazole magnesium a detection wavelength of 290 nm was used, whereas for naproxen the detection wavelength was set at 260 nm. An appropriate calibration curve was applied for quantification of esomeprazole magnesium and naproxen, respectively.

For the quantification and purity determination of esomeprazole magnesium in the solid dosage forms a verified UHPLC method was developed, using an Acquity CSH C18 column (1.7 µm particle size, 2.1 x 100 mm) (Waters, Brussels, Belgium) in an oven set at 40 °C, with a gradient system (30 min) based on the same two-component mobile phase system: aqueous 10 mM ammonium acetate (A) and acetonitrile (B). The gradient used here was: a linear ramp from 2 to 20 min going from 90 % A + 10 % B to 5 % A + 95 % B, holding this condition for 5 min and afterwards changing over to chromatographic start conditions 90 % A + 10 % B from 25 to 25.1 min, maintaining this condition for 4.9 min as an equilibration step preceding a next injection. The flow rate was set at 0.35 ml/min, an injection volume of 1.2 µl was used. For the quantification of esomeprazole magnesium a photo-diode array detector (Acquity, Waters, Brussels, Belgium), with a detection wavelength set at 301 nm, was used. Sample preparation was performed by stirring the extrudates in a 10 ml flask filled with demineralized water : acetonitrile in a 1:1 ratio. An appropriate calibration curve was applied for quantification of esomeprazole magnesium.

The UHPLC system consisted of an isocratic solvent pump, an automatic autosampler and a column oven (Acquity, Waters, Brussels, Belgium). Peak integration and data acquisition was performed using the software package Empower® (Waters, Brussels, Belgium).

Modulated differential scanning calorimetry

The crystallinity of naproxen in the enteric core matrix and the thermal behavior of pure compounds, physical mixtures and corresponding extrudates were studied using a differential scanning calorimeter Q2000 V24.8 equipped with a refrigerated cooling system (RCS) (TA Instruments, Leatherhead, UK). Nitrogen was used as purge gas through the DSC cell (50 ml/min) and the RCS unit (300 ml/min). Samples (± 8 mg) were run in hermetically closed Tzero pans with perforated lid, supplied by TA Instruments, with an underlying heating rate of 2 °C/min. The modulation period and amplitude were set at 60 s and 0.318 °C, respectively. After a first heating cycle to 175 °C, samples were cooled to -30 °C using a linear cooling rate of 10 °C/min. Finally, a second modulated heating cycle was applied. Mass of sample pan and empty reference pan were taken into account. Temperature and enthalpy calibration were performed using an indium standard, whereas calibration of the heat capacity was performed using a sapphire standard. Modulated differential scanning calorimetry (MDSC) data were analyzed using the TA Instruments Universal Analysis 2000 V4.7A software. Melting enthalpies and glass transition temperatures were determined in the total heat flow and reversing heat flow signal, respectively. Reported glass transition temperatures of the physical mixtures were determined in the second heating cycle to ensure maximal interaction between the compounds and to simulate a thermal history comparable to the extrudates when analyzed during the first heating cycle in MDSC. The

degree of crystallinity was calculated comparing the melting enthalpy of the naproxen melting peak in the analyzed sample to that of pure naproxen (147.2 J/g).

X-ray diffraction

Crystallinity was analyzed using X-ray diffraction (XRD) on pure compounds, physical mixtures and corresponding extrudates. X-ray diffraction was performed on a D5000 diffractometer with Cu Kα radiation (λ = 1.54 Å) (Siemens, Karlsruhe, Germany) and a voltage of 40 mV in the angular range (2θ) varying from 4 to 60 ° using a step scan mode with a step size of 0.02 ° and a measuring time of 1 s/step.

Attenuated total reflection Fourier-transform infrared analysis

Attenuated total reflection Fourier-transform infrared (ATR FT-IR) spectroscopy was performed on the pure substances, physical mixtures and extrudates to identify molecular interactions formed between naproxen and the enteric polymers during extrusion. Spectra were recorded in absorbance mode using a Nicolet iS5 ATR FT-IR spectrometer (ThermoFisher Scientific, Karlsruhe, Germany). A diamond ATR crystal was pressed against the samples in order to obtain the ATR FT-IR spectra in the 4000 – 550 cm^{-1} range, with a resolution of 4 cm^{-1}, averaged over 32 scans.

Stability study

Clear core extrudates formulated with different polymer matrices and 30 % naproxen (Table 1), and core extrudates containing 50 % naproxen and 50 % HPMC-AS-LF were manufactured to perform a stability study. Immediately after extrusion, the formulations were filled in an

amber glass container and stored in closed condition at 25 °C/60 %RH and in open and closed condition at 40 °C/75 %RH. To investigate the influence of storage MDSC, XRD, and *in vitro* drug release tests were performed on the extrudates immediately after manufacturing (T0), after 1 week (T1w), 2 weeks (T2w), 1 month (T1m) or 6 weeks (T6w), 3 months (T3m) and 6 months (T6m) storage.

RESULTS AND DISCUSSION

In order to formulate a core/coat fixed-dose combination product via co-extrusion both layers were first independently developed. Afterwards the compatibility of the core and coat matrices was checked. Finally it was evaluated if the final drug-loaded formulations were compatible and if a fixed-dose combination product with the desired release characteristics could be manufactured via co-extrusion.

Core formulation

To develop a core matrix formulation providing enteric protection for naproxen, using hot-melt extrusion (HME) as production technology, three enteric polymers were compared: methacrylic acid - ethylacrylate copolymer (Eudragit® L100-55), hypromellose acetate succinate (HPMC-AS-LF) and hypromellose phthalate (HPMCP-HP-50). Hot-melt extrusion of these polymers in combination with 15 % naproxen required a plasticizer as without plasticizer the torque values during extrusion were too high. Although naproxen (with a melting point at 156.1 °C and a glass transition temperature (*Tg*) of 6.2 °C [10]) had a concentration-dependent plasticizing effect on these polymers (Table 2), the effect of this polymer/drug interaction on the process temperature and/or torque during extrusion cannot be exploited to its full extent as the plasticizing effect was only evident during the second heating cycle of the MDSC analysis of a physical mixture. Hence, these drug/polymer interactions were only established after intense intermolecular contact following the 1st heating phase of the MDSC experiment.

Formulation	*Tg* (° C)		
	Eudragit®L100-55 + 10%TEC	HPMC-AS-LF + 10%TEC	HPMCP-HP-50 + 10%TEC
100% matrix	90.2	91.1	95.7
85% matrix + 15% naproxen	61.4	63.4	59.0
70% matrix + 30% naproxen	24.9	24.2	26.9

Table 2. Glass transition temperatures (*Tg*) of placebo and drug-loaded (15 and 30 %) physical mixtures measured by MDSC in a 2nd heating cycle.

TEC was an efficient plasticizer for Eudragit® L100-55, reducing its *Tg* from 117.7 °C to 108.5 and 90.2 °C after the 1st and 2nd heating cycle, respectively, at a concentration of 10 % TEC. For this formulation a screw speed of 120 rpm and a higher processing temperature at the die-end of the barrel was required to reduce die swell. The addition of 10 % talc to the formulation was critical as it improved the flow properties of the powder, ensuring consistent feeding of the powder into the extruder. When 10 % TEC was added to HPMC-AS-LF the *Tg* lowered from 122.8 °C to 97.5 and 91.1 °C after the 1st and 2nd heating cycle respectively. This formulation, containing 15 % naproxen, yielded an extrudate with a smooth appearance and without die swell when processed at 150 °C. The addition of 10 % TEC to HPMCP-HP-50 as enteric polymer reduced *Tg* from 142.1 °C to 126.6 and 95.7 °C after the 1st and 2nd heating cycle, respectively. The 15 % drug-loaded formulation yielded extrudates that were processable at 145 °C, but had an irregular surface.

At a 15 % naproxen content hot-melt extrusion of all polymer formulations resulted in clear extrudates with the entire drug content molecularly dispersed in the polymer matrix. A higher drug load (30 %) resulted in opaque formulations with a significant degree of crystallinity. However, the extrusion temperature was critical to the physicochemical state of the drug in the extrudates as nearly the entire naproxen content was molecularly dispersed

in the polymer matrices when processed at a higher temperature (Table 1), e.g. HPMC-AS-LF mixtures with 30 % drug processed at 100 and 120 °C contained 29.0 and 2.6 % crystalline drug, respectively. This was also reflected in the X-ray diffractogram of the Eudragit® L100-55 extrudates processed at different extrusion temperatures (Fig. 1).

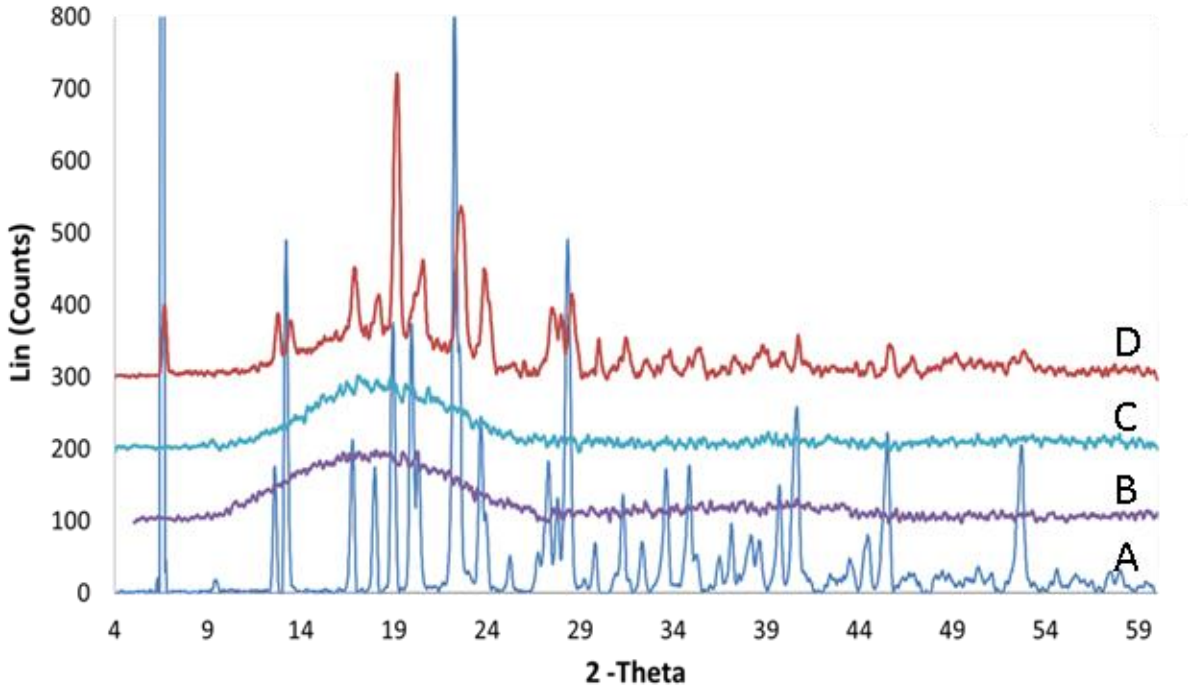

Figure 1. X-ray diffraction patterns of (from bottom to top): naproxen (A), Eudragit® L100-55 (B), the formulation containing 30 % naproxen in a 70 % (Eudragit® L100-55 : TEC 9:1) matrix, processed at, from feed opening to die-end, 110/110/110/110/125/125/125 °C (C) and 100/100/100/100/110/110/110 °C (D).

Interestingly the hypromellose-based polymers containing a higher naproxen content could be extruded at a lower temperature, even when they contained a significant crystalline drug fraction. This can be linked to the plasticizing effect of naproxen on these polymers: thermal processing of mixtures with a higher drug content induced more interaction between drug and polymer in the extrusion barrel. Hence, a lower extrusion temperature could be employed, without risking too high torque values. This plasticizing effect of naproxen was even more evident for a HPMC-AS-LF formulation containing 50 % naproxen which could be

processed without plasticizer at an extrusion temperature of 120/120/120/110/110/100/100 °C from feed opening to die-end, despite its high percentage of crystalline drug.

The *in-vitro* naproxen release profiles of the different polymer formulations containing 10 % TEC as a plasticizer and loaded with 15 % naproxen are shown in Fig. 2. For all formulations naproxen release in 0.1 N HCl was prevented for 2 h. In pH 6.8 buffer HPMCP-HP-50 matrices showed a faster release rate compared to HPMC-AS-LF and Eudragit® L100-55 formulations.

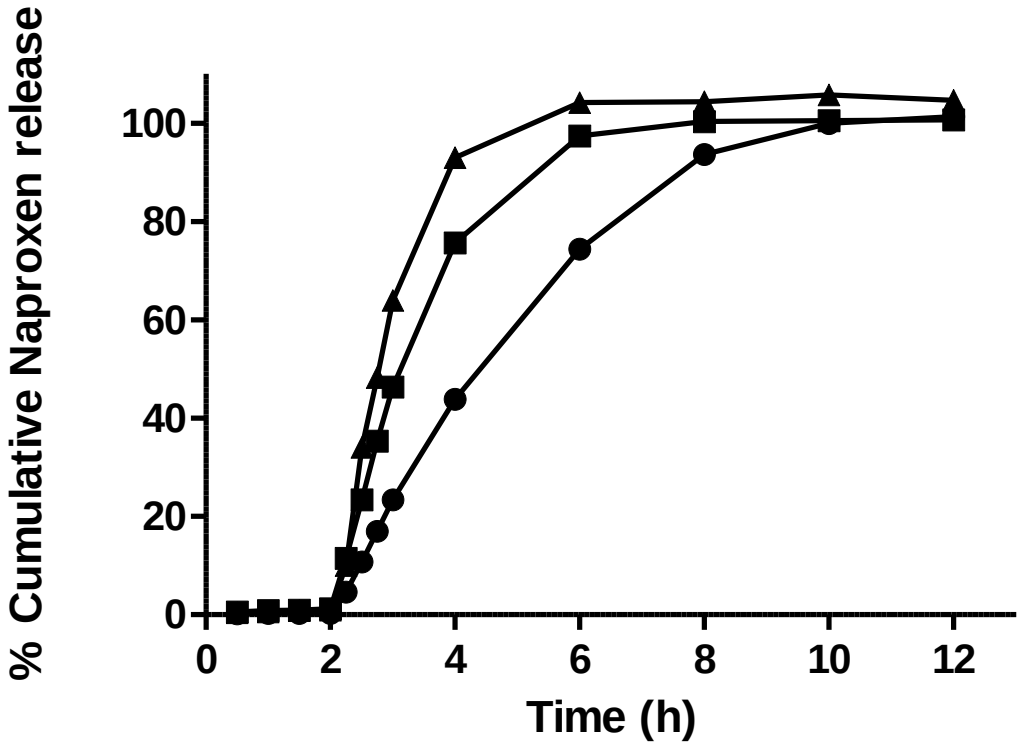

Figure 2. *In-vitro* naproxen release profile of formulations containing 15 % naproxen and an enteric polymer, plasticized with 10 % TEC: Eudragit® L100-55 (●), HPMC-AS-LF (■), HPMCP-HP-50 (▲). Dissolution in 0.1 N HCl (2 h) and pH 6.8 buffer (10 h) at 37 °C using paddle dissolution system at 100 rpm (Mean ± SD; n=3).

Although the process temperature did affect the API's physicochemical state for the 30 % naproxen formulations, it did not have a significant effect on the release profiles. The enteric protection of naproxen during a 2 h period was not impaired in formulations containing 30

and 50 % drug. However, naproxen release in pH 6.8 buffer was determined by drug concentration: after 2 h HPMC-AS-LF matrices containing 30 and 50 % naproxen released 58 and 81 % of their drug content, respectively (Fig. 3).

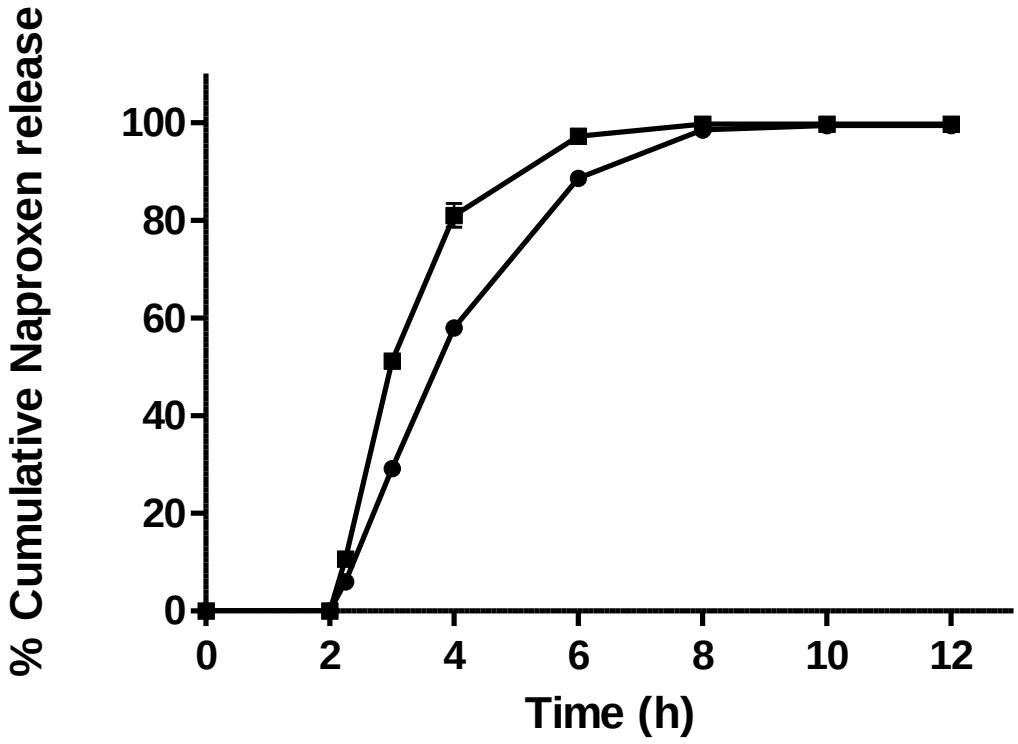

Figure 3. *In-vitro* naproxen release profile of two extruded HPMC-AS-LF formulations with 30 (●) and 50 (■) % drug load. Dissolution in 0.1 N HCl (2 h) and pH 6.8 buffer (10 h) at 37 °C using paddle dissolution system at 100 rpm (Mean ± SD; n=3).

A stability study was performed on the transparent extrudates containing 30 % naproxen and 10 % TEC. Independent of the matrix polymer, naproxen completely recrystallized after two weeks storage at 40 °C/75 %RH, while XRD analysis detected no recrystallization over a 6 month period in a hypromellose matrix stored at 25 °C/60 %RH. For the 50 % naproxen formulation in HPMC-AS-LF the physicochemical state nor the dissolution profiles of the drug had changed after 6 months storage at the different storage conditions.

To identify intermolecular interactions between naproxen and polymer (Eudragit® L100-55, HPMC-AS-LF), Fourier-transform infrared (FT-IR) spectra were collected of transparent extrudates, containing 30 % drug and plasticized with 10 % TEC, immediately after manufacturing and after 2 weeks storage at 40 °C/75 %RH in open condition (i.e. after recrystallization of the drug). From the FT-IR spectra of the Eudragit® L100-55 formulation shown in Fig. 4 and Fig. 5 it is suggested that naproxen is mainly molecularly dispersed in the formulation immediately after processing, since some of the peaks characteristic for naproxen completely disappeared, e.g. peaks at 1347 cm^{-1} (rocking of OH of the carboxyl group [11]) and 642 cm^{-1} (wagging of OH of the carboxyl group [11]), while others broadened, e.g. peaks at 1416 cm^{-1} (in-plane bending of CH of the naphthalene ring [11]) and 1628 cm^{-1} (bond stretching of the naphthalene ring [11]), confirming the loss of crystalline material (1.3 %).

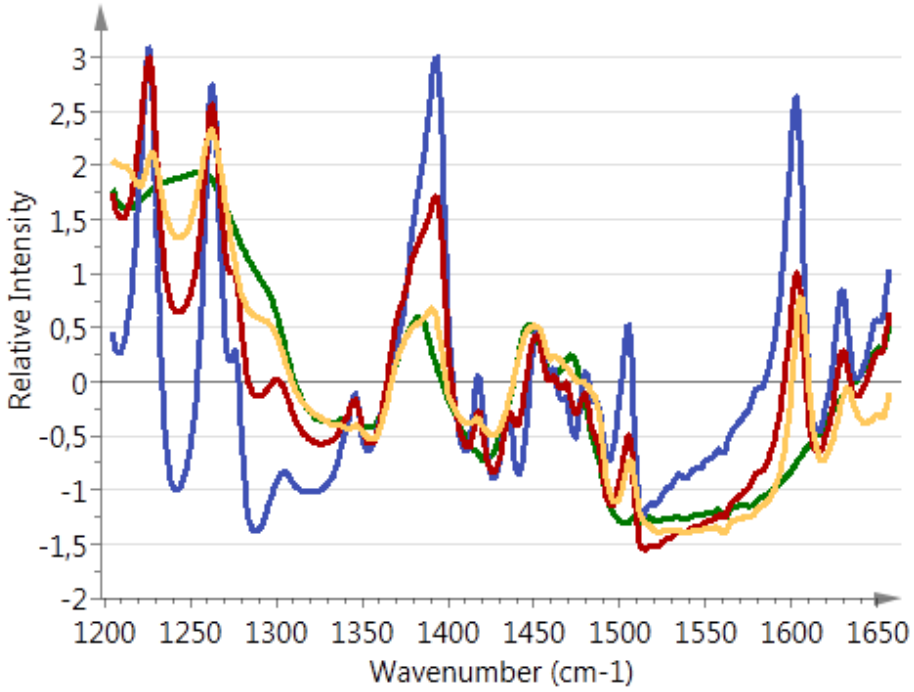

Figure 4. ATR FT-IR spectra of naproxen (blue), Eudragit® L100-55 (green), physical mixture of Eudragit® L100-55 plasticized with 10 % TEC and a drug load of 30 % naproxen (red) and the extrudate of the same formulation immediately after processing (yellow).

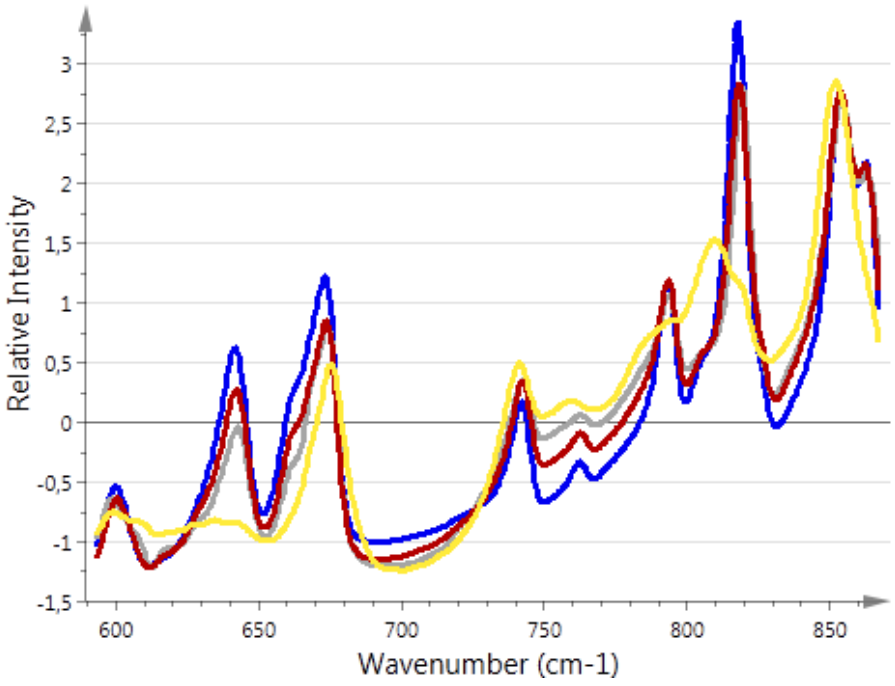

Figure 5. ATR FT-IR spectra of naproxen (blue), physical mixture of Eudragit® L100-55 plasticized with 10 % TEC and a drug load of 30 % naproxen (red) and the extrudate of the same formulation immediately after processing (yellow) and after storage for 2 weeks at 40°C/75%RH (grey).

After storage for 2 weeks at 40 °C/75 %RH the visual recrystallization in the extrudate was confirmed by the appearance of characteristic naproxen peaks in the FT-IR spectra (Fig. 5). The changing ratio between the peaks at 1727 cm^{-1} and 1686 cm^{-1} (attributed to non-hydrogen and hydrogen bonded –C=O stretching of the crystal structure [12]) before and after storage implied that the amount of hydrogen bonds formed during processing between the drug and the matrix decreased over time (Fig. 6). While the interaction between drug and polymer is maximal immediately after processing, the reduction of the peak at 1686 cm^{-1} after storage clearly indicated that the amount of hydrogen bonds decreased. The peak shifts observed in the extrudates for the naproxen peaks at 1227 cm^{-1} (stretching of CO of the methoxy group [11]) and 1603 cm^{-1} (bond stretching of the naphthalene ring [11]) are another indication of the hydrogen bond interaction between drug and matrix (Fig. 4).

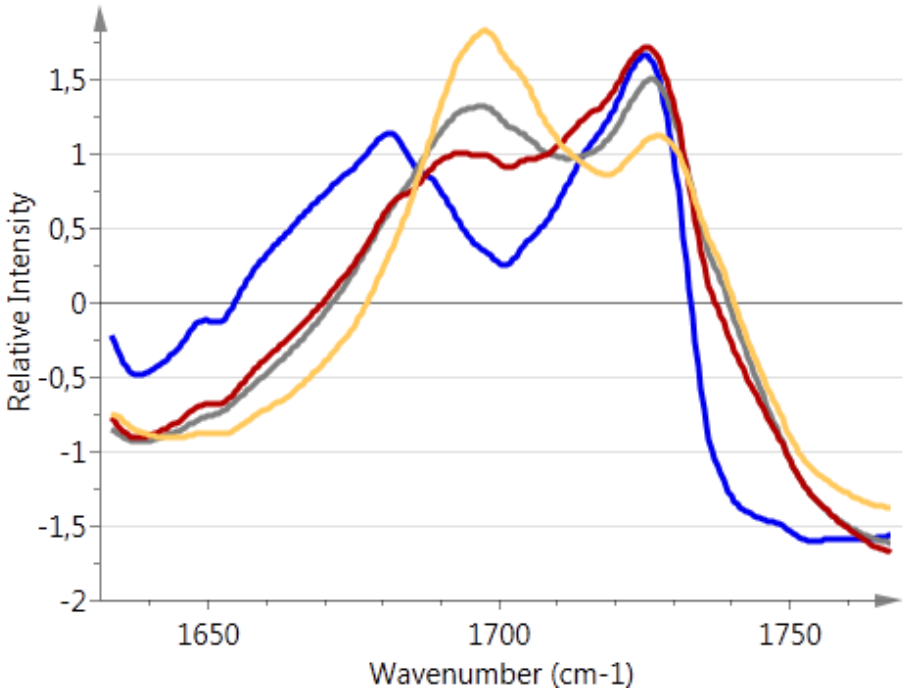

Figure 6. ATR FT-IR spectra of naproxen (blue), physical mixture of Eudragit® L100-55 plasticized with 10 % TEC and a drug load of 30 % naproxen (red), the extrudate of the same formulation immediately after processing (yellow) and after storage for 2 weeks at 40°C/75 %RH (grey).

Also the HPMC-AS-LF formulations showed a partial recrystallization over time. The characteristic naproxen peaks were more pronounced in the FT-IR spectra of the extrudates after storage. Moreover after storage the characteristic naproxen peaks in the FT-IR spectra of the extrudate were not different from those of pure naproxen (Fig. 7). Also at 1727 and 1686 cm^{-1} (Fig. 8) the FT-IR spectrum of the stored extrudate has the same profile as pure drug. This indicated that there is no permanent interaction between HPMC-AS-LF and naproxen.

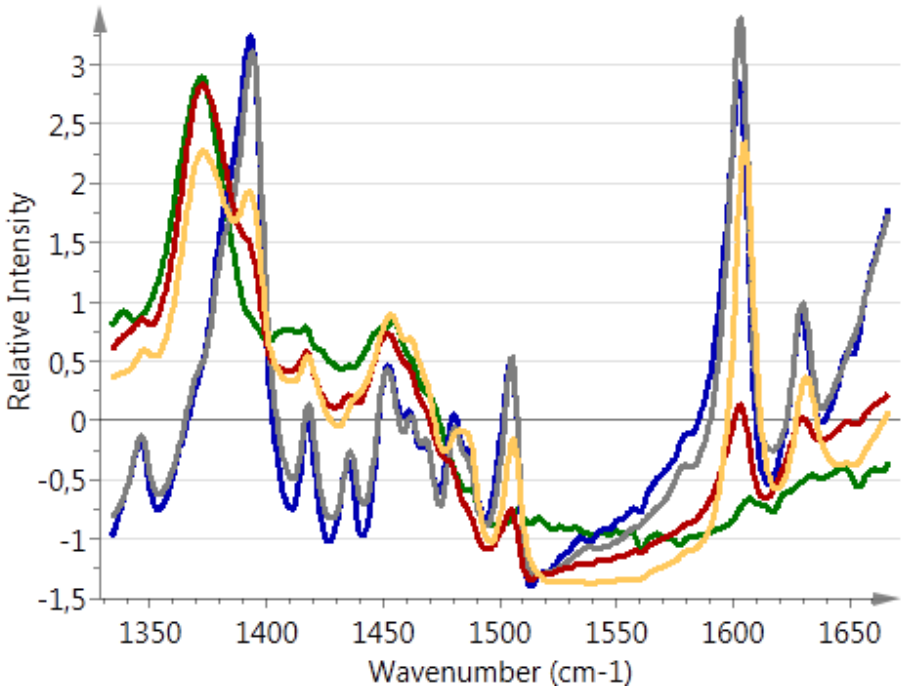

Figure 7. ATR FT-IR spectra of naproxen (blue), HPMC-AS-LF (green), the physical mixture of HPMC-AS-LF plasticized with 10 % TEC and a drug load of 30% naproxen (red), the extrudate of the same formulation immediately after processing (yellow) and after storage for 2 weeks at 40°C/75%RH (grey).

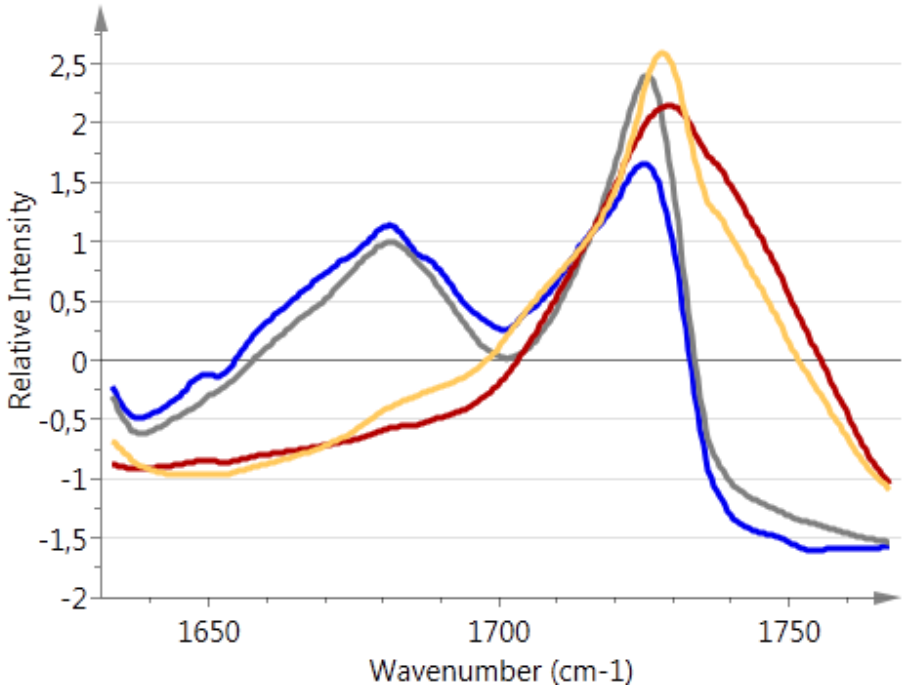

Figure 8. ATR FT-IR spectra of naproxen (blue), physical mixture of HPMC-AS-LF plasticized with 10 % TEC and a drug load of 30 % naproxen (red), the extrudate of the same formulation immediately after processing (yellow) and after storage for 2 weeks at 40°C/75%RH (grey).

Coat formulation

Formulation of esomeprazole magnesium presents a challenge since degradation of the drug can occur due to a high process temperature and in acidic environment [13]. When formulating esomeprazole magnesium in the enteric polymers Eudragit® L100-55, HPMC-AS-LF and HPMCP-HP-50 complete degradation of the drug occurred, most likely due to the presence of acidic groups in the polymers. Therefore esomeprazole magnesium was formulated in an immediate release polymer, separated from the naproxen-containing enteric layer. A similar approach was used in a commercially available combination product of naproxen and esomeprazole magnesium (Vimovo®) which is formulated as an enteric-coated naproxen tablet with a non-enteric-coated esomeprazole magnesium layer on top (both layers are physically separated via a barrier coat). The immediate release polymers tested were PEO 100K, Kollidon®12 PF, Klucel® EF and Methocel® E3. While extrusion of the PEO 100K formulation was feasible at a process temperature of 100 °C, the other polymers required a processing temperature of 130 °C, even with the addition of a plasticizer, and as a result more esomeprazole magnesium degradation occurred: only 40 to 75 % of the drug content was recovered after extrusion, vs. 94 % drug recovery in the PEO 100K formulation. As drug release from the PEO 100K polymer was limited to 70 % after 45 min, PEG 4K was added to the mixtures: complete drug release was observed after 45 min in combination with smooth processing (lower torque) for a 2 % esomeprazole magnesium loaded PEO 100K : PEG 4K (1:1) formulation (Fig. 9).

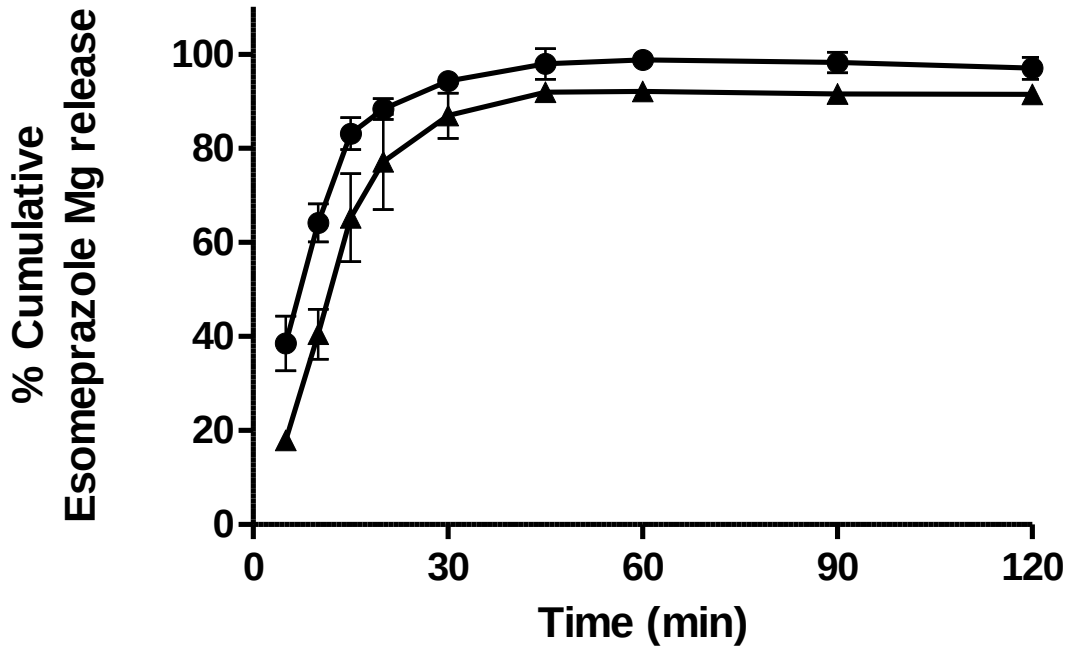

Figure 9. *In-vitro* esomeprazole Mg release from the coat extrudate containing 2 % esomeprazole Mg formulated in PEO 100K : PEG 4K 1:1 (▲) and pure esomeprazole Mg powder (●). Dissolution in demineralized water for 2 h at 37 °C using paddle dissolution system at 100 rpm (Mean ± SD; n=3).

Thermal analysis of the physical mixture and the extruded formulation only revealed a melting endotherm of PEO 100K and PEG 4K, due to dissolution of the esomeprazole magnesium crystals in molten polymer. While two distinct melting endotherms were detected for the physical mixture, only a single endotherm was visible in the extruded sample, indicating the formation of a single phase system.

Co-extrudate formulation

After evaluating the naproxen-containing enteric layer and the esomeprazole magnesium-containing immediate release layer separately, co-extrusion of 50 % naproxen in the HPMC-AS-LF core and 2 % esomeprazole magnesium in the PEO 100K : PEG 4K 1:1 coat yielded an opaque co-extrudate with a smooth surface. However, after cooling of the co-extrudate

discoloration was observed at the interface of core and coat (Fig. 10), and *in vitro* dissolution revealed that only 72 % of the esomeprazole magnesium content could be recovered, despite the fast and complete dissolution of the PEO/PEG layer.

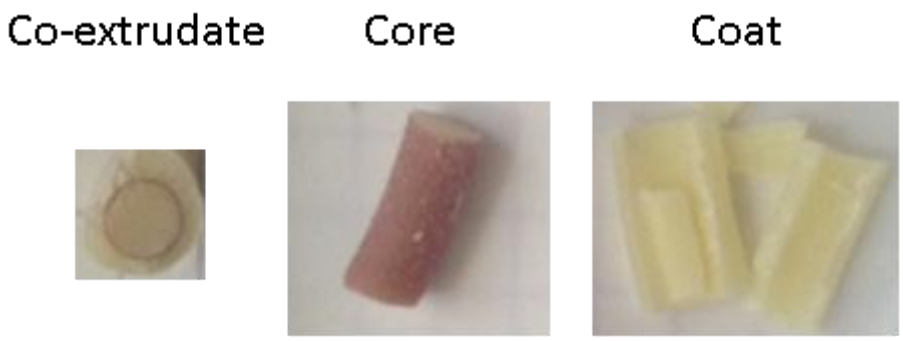

Figure 10. Top view and detail of separated core and coat layer from final co-extrudate, containing 50 % naproxen in the HPMC-AS-LF core and 2 % esomeprazole magnesium in the PEO 100K : PEG 4K 1:1 coat, showing a discoloration at the core surface.

Since this was not seen when processing the co-extrudate with a placebo HPMC-AS-LF + 10 % TEC core, the discoloration is most probably due to an interaction between the naproxen fraction at the core surface and esomeprazole magnesium in the coat, leading to degradation of the acid-labile esomeprazole magnesium. A possible solution to this problem could be the extrusion of a barrier layer between core and coat. This technique is already applied for the production of multilayer films in packaging applications [14, 15], but could not be evaluated at this stage as it implies the use of a third extruder.

CONCLUSION

Hot-melt extrusion was a suitable technique to manufacture an enteric 50 % naproxen-loaded dosage form. Producing a fixed-dose combination product also containing esomeprazole magnesium in a separate immediate releasing coat was not an adequate solution to prevent interaction between both chemically incompatible API's. Co-extrusion as a continuous one-step manufacturing process for the production of a fixed-dose combination product providing enteric release to naproxen and immediate release to esomeprazole magnesium only would be feasible when a third layer of polymer, separating the naproxen-loaded enteric formulation in the core from the coat, would be applied to prevent interaction between both API's.

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GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

The overall aim of this work was to evaluate the possibilities of hot-melt co-extrusion for the production of multilayer oral dosage forms, providing different release profiles for each of the drugs incorporated in the core and coat of the co-extruded form.

Hot-melt co-extrusion was a suitable technique to manufacture mini-matrices with a sustained release metoprolol tartrate-loaded core and an immediate release hydrochlorothiazide-loaded coat. The *in vitro* metoprolol tartrate release from the core could be sustained over a longer period by lowering the concentration of polyethylene oxide (added as a hydrophilic additive to the plasticized ethylcellulose matrix) or by reducing the drug load. The core formulations were successfully combined via co-extrusion with a polyethylene oxide/polyethylene glycol-based coat which provided immediate release of hydrochlorothiazide. The high metoprolol tartrate plasma concentrations detected after oral administration to dogs allowed to reduce the administered dose. In addition, a mathematical model considering the controlled release of metoprolol tartrate from the dosage form and its fate *in vivo* allowed to predict the *in vivo* drug plasma profiles.

Calendering was identified as a downstream processing technique to produce tablet-shaped fixed-dose combination products with an immediate release hydrochlorothiazide-loaded coat and a sustained release metoprolol tartrate-loaded core. The metoprolol tartrate

release from the core of the calendered tablet was significantly prolonged in comparison to a multiparticulate dosage form, prepared via manual cutting of the co-extrudates into mini-matrices, due to longer diffusion path length. The physicochemical properties of both drugs were not affected by the calendering procedure.

Furthermore, a bilayer dosage form which provided sustained release of 2 anti-diabetic drugs was successfully developed via co-extrusion, by incorporating metformin HCl and gliclazide in the core and coat layer, respectively. At least 30 % polycaprolactone was required in the coat layer to adequately sustain the release of the freely soluble metformin HCl from a polycaprolactone core containing 70 % drug. The addition of Kollidon® VA 64 to the polycaprolactone-based coat was essential to ensure complete release of gliclazide over 24 h as the Kollidon® VA 64 phase solubilized the drug in the coat layer.

The final part of this study identified the potential of continuous hot-melt extrusion for the manufacturing of matrix formulations which provided enteric protection to naproxen, using several polymers (Eudragit® L100-55, HPMC-AS-LF and HPMCP-HP-50). These enteric polymer matrices (loaded with naproxen) could be easily processed via co-extrusion in combination with a polyethylene oxide/polyethylene glycol matrix containing esomeprazole magnesium. This co-extruded dosage forms ensured complete release of esomeprazole magnesium within 45 min. However, to prevent interaction between both API's (naproxen and esomeprazole magnesium) at the interface of the core and coat layer a buffering layer between coat and core is required.

Overall, this research project illustrated that hot-melt co-extrusion offers several possibilities for the continuous production of fixed-dose combination products, providing an adequate release profile for each of the drugs incorporated. However, additional topics must be further explored in order to fully develop the possibilities of co-extrusion for the production of oral fixed-dose combination dosage forms and to implement this technique in pharmaceutical industry:

- The downstream process and its effect on product quality should further be investigated to increase the possibilities for in-line shaping or cutting of the co-extrudate. Since most downstream applications require cooling of the melt, the effect of cooling rate on the quality of the extrudate must be elaborated. Next to altering the physicochemical state of the drug, cooling will also change the viscosity of the extrudate, which is particularly relevant for downstream processing.
- New types of polymers, currently not used for oral pharmaceutical applications, should be evaluated for co-extrusion of oral solid dosage forms as there is a growing need for novel polymers, enabling the formulation of combination products via hot-melt co-extrusion. Not only should the materials be thermoplastic and sufficiently deformable after softening, specific characteristics which enable co-extrusion could be also built into the matrix. New tailor-made sets of polymers should be explored in hot-melt co-extrusion. In this way melt viscosity and processing conditions of the different layers could be matched, adequate adhesion between layers could be ensured and specific release characteristics could be achieved.
- An extension of co-extrusion to three- or multilayered co-extrudates would be a logical next step for this innovative technology as this would open new opportunities, e.g. an additional buffering layer could prevent interactions at the interface between

layers, coupling more extruders could allow to combine even more than 2 API's in differently formulated layers.

- For the implementation of a fully equipped hot-melt co-extrusion line for the continuous production of pharmaceutical dosage forms some technical challenges can be identified as this technique is relatively new to the pharmaceutical industry. Some examples of possible improvements are: (a) the incorporation of a robust and accurate system to add a liquid excipient - e.g. plasticizer - to the melt in the extruder; (b) the design of different final product shapes for pharmaceutical fixed-dose combination products; (c) the development of specific downstream equipment allowing in-line shaping or cutting of a co-extrudate without affecting the drug ratio in both layers; (d) the incorporation of PAT in the co-extrusion line, monitoring the properties of the melt, controlling the process settings and measuring the critical quality attributes of the final product.
- Since full-scale manufacturing requires a high throughput rate of materials upscaling from lab-scale extruders to production-scale equipment is an essential aspect for successful introduction of this technique in the pharmaceutical industry. Compensating the impact of upscaling on the flow of the layers through the co-extrusion die and handling the pressure generated in the die would be critical in order to maintain the fixed dimensions of the co-extruded dosage form. To ensure a smooth transition a thorough evaluation of equipment- and process-related parameters and of critical-to-quality attributes would be a first step.
- With respect to the analytical challenges related to fixed-dose combination products extensive compatibility and stability studies would be needed in function of the combination of active pharmaceutical ingredients.

- Additional applications of fixed-dose combination products could be designed by combining co-extrusion with other techniques, e.g. integration of co-extrusion with injection molding could result in the next generation of manufacturing technology for shaping co-extrudates into their final dosage form.
- Combining co-extrusion with other formulation techniques could be promising to tackle specific formulation issues. As hot-melt extrusion can be used for a number of specific applications (e.g. enhancing the solubility of poorly soluble drug compounds, in-situ co-crystal formation, avoiding of particle aggregation, morphological instability and poor wettability via nanoparticle engineering), combining these solutions with co-extrusion can open new perspectives for the formulation of new drug combinations in the increasing market of fixed-dose combination dosage forms.

SUMMARY

In this thesis hot-melt co-extrusion was explored as a manufacturing technique for the production of multilayer pharmaceutical oral dosage forms. Given the increasing importance of fixed-dose combination products in numerous therapeutic fields this innovative continuous manufacturing technology brings significant added value, since the most appropriate matrix can be selected for each of the different drugs incorporated. In this way fixed-dose combination products with enhanced release characteristics can be produced.

In the **Introduction** hot-melt co-extrusion (an innovative technology for the production of pharmaceutical fixed-dose combination dosage forms) is discussed and an overview of the equipment needed for co-extrusion is provided. Because the geometrical design of the die dictates the shape of the final product, different die types are discussed. As shaping of the co-extruded formulation into its final form via a continuous process remains a major challenge, downstream processing of co-extrudates into drug products is discussed. Important requirements for material selection are pointed out and examples of medical and pharmaceutical applications are presented. The increasing importance of fixed-dose combination products is emphasized, given their therapeutic advantages and major contributions to life cycle management. In addition to the benefits of the technique also some barriers to the implementation of co-extrusion in the pharmaceutical industry are discussed.

The successful development via hot-melt co-extrusion of fixed-dose mini-matrices with a core offering a range of controlled release profiles and an immediate release coat was demonstrated in **Chapter 1.** The *in vitro* metoprolol tartrate release from the core was substantially sustained by lowering the concentration of polyethylene oxide (added as a hydrophilic additive to the plasticized ethylcellulose matrix) or by reducing the drug load. The *in vitro* release of hydrochlorothiazide from the polyethylene oxide/polyethylene glycol coat was completed within 45 min for all formulations. Tensile testing of the core/coat mini-matrices revealed an adequate adhesion between both layers. Raman mapping showed that no migration of active substances occurred between core and coat. Physicochemical state characterization indicated that the crystalline state of metoprolol tartrate was not affected by thermal processing via hot-melt extrusion, while hydrochlorothiazide was dissolved in the coat. These physicochemical characteristics were maintained during the stability study. Considering the bioavailability of metoprolol tartrate after oral administration to dogs, the different co-extruded formulations offered a range of sustained release profiles. Moreover, high metoprolol tartrate plasma concentrations were reached in dogs, which allowed to reduce the administered dose. Interestingly, the resulting metoprolol tartrate plasma concentrations could be predicted using an appropriate mathematical model, based on the observed *in vitro* release kinetics and the *in vivo* fate of metoprolol tartrate. The theoretical predictions agreed well with the independent experimental results, implying the number of *in vivo* studies required for product optimisation can be reduced.

In **Chapter 2** calendering was evaluated as a downstream processing step to continuously produce tablet-shaped fixed-dose combination products from multilayered matrix co-extrudates. Cylindrical co-extrudates with a metoprolol tartrate-loaded sustained-release

core and a hydrochlorothiazide-loaded immediate-release coat were shaped in-line via calendering, using chilled rolls with tablet-shaped cavities. *In vitro* metoprolol tartrate release from the ethylcellulose core of the calendered tablets was sustained over 24 to 48 h and was significantly prolonged in comparison to the sustained release of a multiparticulate dosage form, prepared by manual cutting of the co-extrudates into mini-matrices. Analysis of the dosage forms using X-ray micro-computed tomography only detected small differences between the pore structure of the core of the calendered tablet and the mini-matrices. Differences in diffusion path length were the main contributing factor to changes in release kinetics in function of the post-processing technique. Terahertz pulsed imaging visualized that adhesion between the core and coat of the calendered tablet was not complete and a gradient in coat thickness (varying from 200 to 600 µm) was observed. Modulated differential scanning calorimetry and X-ray diffraction indicated that the physicochemical properties of both drugs were not affected by the calendering procedure.

In **Chapter 3** bilayer dosage forms containing an anti-diabetic drug in both core and coat were successfully developed by co-extrusion, offering a fixed-dose combination product sustaining the release over 24 h for both metformin HCl and gliclazide. From this study it was clear that co-extrusion of a coat layer, containing at least 30 % CAPA® 6506 as a hydrophobic polymer, was necessary to adequately sustain the release of the highly dosed freely soluble drug from the CAPA® 6506 core containing 70 % drug. Inclusion of Kollidon® VA 64 in the coat ensured complete release over 24 h of gliclazide via solubilization of the drug in the Kollidon® VA 64 phase.

In **Chapter 4** co-extrusion was evaluated as a manufacturing technique to produce a fixed-dose combination product providing enteric protection to naproxen and immediate release to esomeprazole magnesium. The plasticizing effect of naproxen and triethyl citrate (TEC) was tested on the enteric polymers investigated (Eudragit® L100-55, HPMC-AS-LF and HPMCP-HP-50). Core matrix formulations containing HPMC-AS-LF, TEC and a naproxen load of 15, 30 and 50 % were processed and characterized. *In vitro* naproxen release in 0.1 N HCl was prevented during 2 h for all formulations. Hot-melt extrusion was a suitable technique to manufacture an enteric 50 % naproxen-loaded dosage form. When esomeprazole magnesium was formulated in a polyethylene oxide 100K : polyethylene glycol 4K (1:1) matrix, the formulation could be easily processed and complete *in vitro* drug release was observed after 45 min. A fixed-dose combination product which contained esomeprazole magnesium in the immediate release coat and naproxen in the core was not an adequate solution for the chemical incompatibility between both API's as drug interaction occurred at the interface of both layers. Hence co-extrusion as a one-step manufacturing process of a fixed-dose combination product providing enteric release to naproxen and immediate release to esomeprazole magnesium would only be feasible when an intermediate polymer layer is applied to separate the naproxen-containing enteric core formulation from the esomeprazole magnesium-containing coat.

SAMENVATTING

In deze doctoraatsthesis werd het gebruik van 'hot-melt' co-extrusie onderzocht als productietechniek voor farmaceutische orale toedieningsvormen. Door het toenemende belang van combinatiepreparaten in verschillende therapeutische domeinen levert deze innovatieve en continue technologie een belangrijke toegevoegde waarde. Door de meest geschikte drager te selecteren voor elk van de lagen kunnen combinatiepreparaten met optimale vrijstellingseigenschappen voor elk geneesmiddel aangemaakt worden.

Eerst wordt een **introductie** tot 'hot-melt' extrusie en een overzicht van de benodigde uitrusting gegeven. Omdat het ontwerp van de matrijs de vorm van het eindproduct bepaalt, worden verschillende matrijstypes besproken. Een belangrijke uitdaging blijft om het co-extrudaat in een finale toedieningsvorm aan te maken via een continu proces. Hiervoor wordt een overzicht gegeven van diverse oplossingen. Belangrijke vereisten voor materiaalselectie alsook voorbeelden van medische en farmaceutische toepassingen worden beschreven. Aanvullend op de voordelen van de techniek worden ook enkele knelpunten voor de implementatie van co-extrusie in de farmaceutische industrie aangehaald.

De succesvolle ontwikkeling van minimatrices via 'hot-melt' co-extrusie met een kern die mogelijkheden biedt voor verschillende vertraagde vrijstellingsprofielen en een mantel die zorgt voor snelle geneesmiddelvrijstelling wordt besproken in **Hoofdstuk 1**. De *in vitro* vrijstelling van metoprololtartraat uit de kern werd in belangrijke mate vertraagd door de

concentratie aan polyethyleenoxide, toegevoegd als hydrofiele component aan de matrix bestaande uit ethylcellulose en een weekmaker, te verlagen of door de geneesmiddelconcentratie te verminderen. De *in vitro* vrijstelling van hydrochloorthiazide uit de polyethyleenoxide/polyethyleenglycol mantel was binnen 45 minuten voltooid. Trektesten gaven aan dat er voldoende adhesie was tussen de kern en de mantel. Bovendien toonde Raman 'mapping' aan dat er geen migratie van de activa plaatsvond tussen de kern en de mantel. Vaste toestand karakterisatie wees uit dat de kristallijne toestand van metoprololtartraat behouden bleef tijdens 'hot-melt' extrusie, terwijl het hydrochloorthiazide opgelost was in de mantel. Deze fysicochemische karakteristieken werden bevestigd tijdens een stabiliteitsstudie. Na orale toediening van de verschillende co-extrudaten aan honden werden voor de biologische beschikbaarheid van metoprololtartraat verschillende vertraagde vrijstellingsprofielen waargenomen. Meer nog, de hoge metoprololtartraat plasmaconcentratie die bereikt werd bij de hond, liet toe om de toegediende dosis te halveren. Gebruik makend van een gepast wiskundig model, opgesteld op basis van de waargenomen *in vitro* vrijstellingskinetiek en het *in vivo* plasmacurvetijdsprofiel, kon aangetoond worden dat de theoretische voorspellingen een hoge predictiegraad vertoonden. Hierdoor kan het aantal *in vivo* studies nodig voor productoptimalisatie gereduceerd worden.

In **Hoofdstuk 2** werd 'calendering', als verwerkingsstap om op continue wijze combinatiepreparaten in tabletvorm te produceren, geëvalueerd. Cilindrische co-extrudaten met metoprololtartraat en hydrochloorthiazide werden 'in-line' gevormd met behulp van een 'calender', waarbij gekoelde rollen met tabletvormige uitsparingen werden gebruikt. De *in vitro* metoprololtartraatvrijstelling uit de ethylcellulose kern van de met de 'calender'

gevormde tabletten werd vertraagd over een tijdsspanne van 24 tot 48 u, wat significant trager was dan de vertraagde vrijstelling die werd bekomen met de multiparticulaire toedieningsvormen. Bij analyse van de toedieningsvormen aan de hand van X-stralen micro-CT werden enkel kleine verschillen in poriënstructuur gedetecteerd tussen de kern van het gevormde tablet en de minimatrices. De diffusielengte bleek het belangrijkste vrijstellingsmechanisme te zijn. Door middel van 'terahertz pulsed imaging' werd duidelijk dat de adhesie tussen kern en mantel niet volledig was en werd een diktegradiënt van de mantel (variërend tussen 200 en 600 µm) geobserveerd. Aan de hand van gemoduleerde differentiaal scanning calorimetrie en X-stralen diffractie werd aangetoond dat de fysicochemische eigenschappen van beide geneesmiddelen niet aangetast werden door het 'calenderen'.

In **Hoofdstuk 3** werd aan de hand van co-extrusie een monolithische toedieningsvorm ontwikkeld bestaande uit twee lagen, welke elk een anti-diabeticum bevatten. Het combinatiepreparaat vertoonde een vertraagde vrijstelling over 24 u voor zowel metformine HCl als gliclazide. Uit deze studie werd duidelijk dat co-extrusie van een mantel, met minstens 30 % CAPA® 6506 als hydrofoob polymeer, nodig was om de vrijstelling van het hoog gedoseerde goed wateroplosbare metformine HCl voldoende te vertragen. Het solubiliseren van gliclazide in de Kollidon® VA 64 fase van de mantel verzekerde de volledige vrijstelling van het geneesmiddel over 24 u.

In **Hoofdstuk 4** werd co-extrusie geëvalueerd als productietechniek voor het aanmaken van een combinatiepreparaat dat enterische bescherming biedt aan naproxen en een onmiddellijke vrijstelling aan esomeprazole magnesium. Het weekmakend effect van

naproxen en tri-ethyl citraat (TEC) op de onderzochte enterische polymeren (Eudragit® L100-55, HPMC-AS-LF en HPMCP-HP-50) werd getest. Matrixformulaties voor de kern bestaande uit HPMC-AS-LF, TEC en een naproxen concentratie van 15, 30 of 50 % werden aangemaakt en gekarakteriseerd. De *in vitro* naproxen vrijstelling in 0.1 N HCl werd verhinderd gedurende 2 u voor alle formulaties. 'Hot-melt' extrusie bleek een geschikte techniek om een 50 % naproxen beladen toedieningsvorm aan te maken. Wanneer esomeprazole magnesium geformuleerd werd in een polyethyleenoxide 100K : polyethyleenglycol 4K (1:1) matrix, kon de formulatie makkelijk verwerkt worden en werd een volledige *in vitro* geneesmiddelvrijstelling waargenomen na 45 min. Een combinatiepreparaat aanmaken dat naast naproxen ook esomeprazole magnesium bevat in een afzonderlijke onmiddellijke vrijstellingsmantel bleek geen afdoende oplossing om interactie tussen de twee chemisch onverenigbare geneesmiddelen te vermijden. Co-extrusie als continu productieproces voor het aanmaken van een combinatiepreparaat dat enterische bescherming biedt aan naproxen en onmiddellijke vrijstelling van esomeprazole magnesium toelaat, zou enkel bruikbaar zijn wanneer een derde polymeerlaag de kern van de mantel afschermt.

Er kan geconcludeerd worden dat 'hot-melt' co-extrusie, als continue productietechniek, talrijke mogelijkheden biedt voor het aanmaken van combinatiepreparaten voor oraal gebruik.

CURRICULUM VITAE

PERSONAL INFORMATION

SURNAME:	Vynckier
FIRST NAMES:	An-Katrien Tine Christiane
NATIONALITY:	Belgian
DATE OF BIRTH:	06/06/1980
PLACE OF BIRTH:	Roeselare, Belgium
CIVIL STATUS:	married
ADDRESS:	Titecastraat 3
	8200 Brugge
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EDUCATION

2011 – present:	PhD in Pharmaceutical Sciences: "Co-extrusion as a continuous production process for fixed-dose combination dosage forms" Promoters: Prof. Dr. Jean Paul Remon and Prof. Dr. Chris Vervaet Laboratory of Pharmaceutical Technology, UGent, Belgium
2006 – 2007:	Postgraduate Business Administration Leuven School of Business and Economics, KULeuven, Belgium
2003 – 2004:	Master in Industrial Pharmacy Interacademic cooperation UGent, KULeuven, UA and VUB, Belgium
1998 – 2003:	Master in Drug Development (Pharmacist) KULeuven, Belgium

EXPERIENCE

2011 – present:	Scientist at SEPS Pharma nv Gent, Belgium
2009 – present:	Owner and titular of Apotheek Vynckier Oudenburg, Belgium
2008:	Pharmacist at nv PSB Wespelaar, Belgium
2006 – 2008:	Senior Scientist Drug Product Stability and Specification Development Janssen Pharmaceutica, Beerse, Belgium
2005 – 2006:	Process Engineer Medical Devices Janssen Pharmaceutica, Beerse, Belgium
2004 – 2005:	Industrial Pharmacist internship QA Medical Devices Janssen Pharmaceutica, Beerse, Belgium
2004:	Master thesis: “HPLC analysis of cysteamine-phosphate on ion-exchange columns.” Promotors : Prof. Dr. J. Hoogmartens, Prof. Dr. A. Van Schepdael Laboratory for Pharmaceutical Chemistry and Analysis of Medicins, KULeuven, Belgium
2003:	Master thesis: “Regulated production and isolation of natural human IP-10 (by Interferon-ɣ induced protein-10).” Promotors : Prof. Dr. J. Van Damme, Prof. Dr. P. Proost Laboratory for Molecular Immunology, Rega Institute, KULeuven, Belgium
2002 – 2003:	Pharmacist internship Apotheek De Lindeboom, Mechelen, Belgium
2002:	Pharmacist internship clinical training UZ Gasthuisberg, Leuven, Belgium

PUBLICATIONS IN PEER REVIEWED JOURNALS

Enteric protection of naproxen in a fixed-dose combination product produced by hot-melt co-extrusion.
***A.-K. Vynckier**, M. De Beer, T. Monteyne, J. Voorspoels, T. De Beer, J.P. Remon, C. Vervaet*
International Journal of Pharmaceutics (2015) doi: 10.1016/j.ijpharm.2015.06.010.

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Submitted to Journal of Pharmacy and Pharmacology (2015)

Calendering as a direct shaping tool for the continuous production of fixed-dose combination products via co-extrusion.
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Development of sustained and dual drug release co-extrusion formulations for individual dosing.
*E.J. Laukamp, **A.-K. Vynckier**, J. Voorspoels, M. Thommes, J. Breitkreutz*
European Journal of Pharmaceutics and Biopharmaceutics 89 (2015) 357-364.

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***A.-K. Vynckier**, L. Dierickx, L. Saerens, J. Voorspoels, Y. Gonnissen, T. De Beer, C. Vervaet, J.P. Remon*
International Journal of Pharmaceutics 464 (2014) 65-74.

Hot-melt co-extrusion: requirements, challenges and opportunities for pharmaceutical applications
***A.-K. Vynckier**, L. Dierickx, J. Voorspoels, Y. Gonnissen, J.P. Remon, C. Vervaet*
Journal of Pharmacy and Pharmacology 66 (2014) 167-179.

Capillary electrophoresis method development for determination of impurities in sodium cysteamine phosphate samples.
*A. Carvalho, J. Pauwels, B. De Greef, **A.-K. Vynckier**, W. Yuqi, J. Hoogmartens, A. Van Schepdael*
Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 120-125.

Microbial Toll-like receptor ligands differentially regulate CXCL10/IP-10 expression in fibroblasts and mononuclear leukocytes in synergy with IFN-ɣ and provide a mechanism for enhanced synovial chemokine levels in septic arthritis.
*P. Proost, **A.-K. Vynckier**, F. Mahieu, W. Put, B. Grillet, S. Struyf, A. Wuyts, G. Opdenakker, J. Van Damme*
European Journal of Immunology 33 (2003) 3146-3153.

PRESENTATIONS AT CONFERENCES

Oral presentations

Hot-melt co-extrusion for the production of fixed-dose combination products with a controlled release ethylcellulose matrix core.
A.-K. Vynckier, L. Dierickx, L. Saerens, J. Voorspoels, Y. Gonnissen, T. De Beer, C. Vervaet, J.P. Remon
7th Annual symposium of Pharmaceutical Solid State Research Cluster, Lille (France), 4-6 July 2013.

Hot-melt co-extrusion for the production of fixed-dose combination products with a controlled release ethylcellulose matrix core.
A.-K. Vynckier, L. Dierickx, L. Saerens, J. Voorspoels, Y. Gonnissen, T. De Beer, C. Vervaet, J.P. Remon
IDEA Interreg4A-2seas Project Closing Conference, Lille (France), 20-21 June 2013.

Hot-melt co-extrusion for the production of fixed-dose combination products with a controlled release ethylcellulose matrix core + Demo co-extrusion
A.-K. Vynckier, L. Dierickx, J. Voorspoels, Y. Gonnissen, C. Vervaet, J.P. Remon
5th IDEA Interreg4A-2seas meeting and training day, Gent (Belgium), 8-9 November 2012.

Hot-melt extrusion
A.-K. Vynckier, J. Voorspoels
4th IDEA Interreg4A-2seas meeting, Gent (Belgium), February 23rd 2012.

Poster presentations

Hot-melt extrusion for the production of an enteric matrix formulation of naproxen.
A.-K. Vynckier, J.P. Remon, C. Vervaet
Knowledge for growth, Gent (Belgium), May 21st 2015.

Hot-melt extrusion for the production of an enteric matrix formulation of naproxen.
A.-K. Vynckier, J. Voorspoels, Y. Gonnissen, J.P. Remon, C. Vervaet
Poorly soluble drugs workshop, Lille (France), July 2nd 2014.

Sustained release of a high-dosed water-soluble compound in a co-extruded fixed-dose combination product.
A.-K. Vynckier, J. Voorspoels, Y. Gonnissen, J.P. Remon, C. Vervaet
Knowledge for growth, Gent (Belgium), May 8th 2014.

Calendering as a direct shaping tool for the continuous production of fixed-dose combination products via co-extrusion.
A.-K. Vynckier, H. Lin, J.A. Zeitler, J.-F. Willart, E. Bongaers, J. Voorspoels, Y. Gonnissen, J.P. Remon, C. Vervaet
Knowledge for growth, Gent (Belgium), May 8th 2014.

Calendering as a direct shaping tool for the continuous production of fixed-dose combination products via co-extrusion.
A.-K. Vynckier, H. Lin, J.A. Zeitler, J.-F. Willart, E. Bongaers, J. Voorspoels, Y. Gonnissen, J.P. Remon, C. Vervaet
9th PBP World Meeting, Lisbon (Portugal), March 31st - April 3rd 2014.

Hot-melt co-extrusion for the production of fixed-dose combination products with a controlled release ethylcellulose matrix core
A.-K. Vynckier, L. Dierickx, J. Voorspoels, Y. Gonnissen, T. De Beer, C. Vervaet, J.P. Remon
Knowledge for growth, Gent (Belgium), May 30th 2013.

Hot-melt co-extrusion for the production of fixed-dose combination products with a controlled release ethylcellulose matrix core
A.-K. Vynckier, L. Dierickx, J. Voorspoels, Y. Gonnissen, T. De Beer, C. Vervaet, J.P. Remon
Meeting of the Belgian-Dutch Biopharmaceutical Society, Utrecht (The Netherlands), November 9th 2012.

Hot-melt co-extrusion for the production of fixed-dose combination products with a controlled release ethylcellulose matrix core
A.-K. Vynckier, L. Dierickx, J. Voorspoels, Y. Gonnissen, T. De Beer, C. Vervaet, J.P. Remon
2012 AAPS annual meeting and exposition, Chicago (USA), 14-18 October 2012

ATTENDED COURSES, CONFERENCES AND WORKSHOPS

- *Workshop: APS Amorphous IV: Hot-melt extrusion and powder technology in pharmaceutical industry, University of Greenwich (United Kingdom), 12-13 June 2012.*
- *Conference: 8th PBP World Meeting, Istanbul (Turkey), 19-22 March 2012*
- *Course: Differential Scanning Calorimetry (DSC) Practical training course, TA Instruments, Zellik (Belgium), December 14th 2011.*
- *Course: Modulated Differential Scanning Calorimetry (MDSC) Theoretical training course, TA Instruments, Gent (Belgium), November 30th 2011.*
- *Course: Multivariate Data Analysis, UGent, Gent (Belgium), November 2011.*

- *Course: Differential Scanning Calorimetry (DSC) Theoretical training course, TA Instruments, Zellik (Belgium), 15-16 November 2011.*
- *Workshop: 4th International symposium on pharmaceutical melt extrusion Evonik, Frankfurt (Germany), 9-10 November 2011.*
- *Course: Design of Experiments, UGent, Gent (Belgium), October 2011.*
- *Workshop: Poorly soluble drugs workshop, Lille (France), September 15th 2011.*
- *Conference: 3rd IDEA Interreg4A-2seas meeting, Lille (France), 7-8 September 2011*
- *Workshop: Pharmaceutical Extrusion Seminar Leistritz, Clinton, NJ (USA), 15-16 June 2011.*

LANGUAGE SKILLS

	Speaking	Reading	Writing
Dutch (native language)	very good	very good	very good
English	very good	very good	very good
French	good	good	good